PAPER • OPEN ACCESS

Advance on the preparation technology and antihyperlipidemia mechanism of phytosterols

To cite this article: Chuanhan Deng 2020 IOP Conf. Ser.: Earth Environ. Sci. 615 012107

View the article online for updates and enhancements.

You may also like

Rosyada et al.

- <u>Separation of squalene rich fraction from</u> palm oil fatty acid distillate (*PFAD*): A review R P Nurfatimah, K G S Ahmadi, I Hapsari
- et al.
- <u>Utilization of spirulina as functional food:</u> <u>Phytosterol and amino acid profiles study</u> D Fithriani and E Sinurat
- <u>Phytochemicals screening and anti-oxidant</u> activity of hydroethanolic extracts of <u>Ruellia tuberosa L</u> Anna Safitri, Anna Roosdiana, Istoria

The Electrochemical Society Advancing solid state & electrochemical science & technology



DISCOVER how sustainability intersects with electrochemistry & solid state science research



This content was downloaded from IP address 3.147.89.85 on 05/05/2024 at 08:13

IOP Conf. Series: Earth and Environmental Science 615 (2020) 012107 doi:10.1088/1755-1315/615/1/012107

Advance on the preparation technology and antihyperlipidemia mechanism of phytosterols

Chuanhan Deng

College of pharmacy, China pharmaceutical university, Nanjing City, Jiangsu Province, China

2020171692@stu.cpu.edu.cn, chuanhandeng@qq.com

ABSTRACT: Phytosterols is a kind of natural active substance, which has many physiological functions that beneficial to human body. It can be used as pharmaceutical raw materials and functional food supplements. In recent years, phytosterols have been extensively studied in the prevention of cardiovascular diseases, and a series of research results have been reported. This paper combined the literature recent decades, and introduced the source and distribution, structures and properties of phytosterol, focusing on the preparation technology of phytosterol, including solvent extraction, esterification, distillation, supercritical CO2 extraction, saponification method and microorganism fermentation, and the mechanism of blood lipid lowering function of phytosterols were discussed and summarized. The future research direction was prospected in order to provide the reference for the development and utilization of phytosterols.

1. INTRODUCTION

Phytosterols are important structural components that occur in the cells of plants, animals and microorganisms. Their structure is perhydrocyclopentanophenanthrene (Fig. 1[1]). The skeleton is often connected with hydroxyl in C3, which can form derivatives via an ester or glycosidic bond. There are junction points connecting other carbon chains, which define the properties and functions of phytosterol molecules.[2] Phytosterol is a white solid powder with a melting point of more than 100°C and a high boiling point. The melting point is 139~142°C of β-sitosterol, 157~158°C of campesterol, and 165~167°C of stigmasterol. The boiling point is 501.9°C of β-sitosterol, and 490.4°C of stigmasterol. Phytosterols can be dissolved in organic solvent like ether, petroleum ether, benzene, trichloroethane and ethyl acetate, and cannot be dissolve in water. The density is a little large than water, and the relative molecular mass is from 386 to 456. [3]



Figure 1 Chemical structure of the phytosterol



Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI. Published under licence by IOP Publishing Ltd

IOP Publishing

Phytosterols exist universally in the roots, stems, foliage, fruits and seeds of various species of plants. Not only do they have the therapeutic effects of lowering serum cholesterol levels[4], but also have effects of anti-inflammatory, analgesia, preventing cardiovascular and cerebrovascular diseases, antioxidation, antineoplastic therapy[5], immunoregulation, skin protection and delaying senescence. Hence researches and exploitations to them are always hotspot in nature products researching field. In recent years, they have been widely used in medicine, food, cosmetics and other fields as drug intermediates and functional active factors.

2. PREPARATION TECHNOLOGY OF PHYTOSTEROL

2.1 Solvent Extraction

EExtraction is a traditional method that usually used in massive commercial process because of the simplicity, yet the disadvantages are large consume of solvent, environment contamination and potential safety hazard. This method had been well developed in extracting phytosterol many years ago. Lin[6] used the mixture of methanol and acetone to extract phytosterol from deodorant distillates. With the process of crystallization in -20°C, centrifugation and filtration, the extraction efficiency reached to 80%. Robinson[7] extracted phytosterol from unsaponifiable matter of tall oil using mixed heptane and water. The ratio was 65.9%, and the purity reached to 94.3%. Some studies have found that the sterol components extracted with different solvents are quite special. Hamunen[8] extracted phytosterol from unsaponifiable matter of vegetable oil by methanol, 65%~80% of sterol was sitosterol.

With the development of microwave and ultrasonic technology, many researchers used them as an auxiliary method for solvent extraction. Wang[9] extracted sterol from pumpkin seeds with ethyl acetate as solvent by ultrasonic-assisted method. The time was shortened to 50min after optimizing the process conditions, and the yield was 1.106mg/g. Xu[10] extracted phytosterol from white mulberry bark with ethyl acetate by microwave - assisted method. Compared with solvent extraction method and ultrasonic method, the yield of microwave-assisted method increased by 1.57% and 19.07% respectively, and the extraction time was greatly shortened to 41s.

2.2 Esterification and Distillation

The esterification and distillation method is used to extract from deodorant distillate. The raw material was pretreated with esterification to convert free fatty acids into fatty acid methyl ester with lower boiling point. Then methyl ester and vitamin E were removed using molecular distillation. Phytosterols were finally obtained after adding alkali to remove glycerol ester.[11] Take soybean deodorization distillate as an example, deodorization distillates mainly contain free fatty acids, sterols, vitamin E, sterols esters, glycerides, some odor substances and pigments.[12] Under high vacuum, the substances with lower boiling point than sterol were evaporated, and sterols are concentrated in heavy phase. Most of deodorization distillates had lower boiling points than sterol. The boiling point is 350°C~360°C of oleic acid, 218.5°C of methyl oleate, 200°C~220°C of vitamin E.

In the stage of esterification pretreatment, chemical catalysis was generally adopted in industry, with concentrated sulfuric acid as catalyst, methanol or ethanol as reactant, and esterification reaction occurs by heating reflux.[13] This method was low cost, but it pollutes the environment.

In recent years, many scholars were exploring a new method of environmental protection enzymatic method, which means using lipase to catalyze free fatty acid esterification to form fatty acid methyl ester. This approach was mild, safe and pollution-free, but the disadvantages were the high price of enzymes and the special requirements for the composition and physical and chemical properties of raw materials.

After the esterification pretreatment reaction, molecular distillation, a technology to separate materials in liquid-liquid state under high vacuum by making use of the difference of free path of molecular motion of different substances, was a good method to separate the deodorant distillates. The separation temperature was lower than the boiling point of the material, so it was especially suitable

IOP Conf. Series: Earth and Environmental Science 615 (2020) 012107 doi:10.1088/1755-1315/615/1/012107

for the separation of high boiling point and heat sensitive material.[14] Suresh[15] used the immobilized non-specific lipase SP-382 to catalyze the free fatty acids in the deodorization distillate. The esterification rate was as high as 96.5%. Then remove methyl ester by vacuum distillation, recovery of sterol and vitamin E step by step, the final extraction rate of sterol and vitamin E were more than 90%.

2.3 Supercritical CO2 Extraction

Supercritical CO2 extraction means that the raw material forms a high-pressure fluid mixture with supercritical CO2 by controlling temperature and pressure, then different components were separated according to the difference of molecular weight, polarity and boiling point [16]. Janet[17] extracted sterol from corn oil, rapeseed oil, cottonseed oil, soybean oil and rice bran oil respectively by supercritical CO2 extraction, with good results that the content of sterol in corn oil was 6.8 mg/g, and that in supercritical fluid extraction was concentrated to 153.5 mg/g. Hrabovski[18] extracted the crude oil from pumpkin seeds by n-hexane, petroleum ether and supercritical CO2 extraction then analyzed their sterol content. The crude oil yield of n-hexane and petroleum ether extraction was 43.37% and 44.65%, respectively, which were higher than that of supercritical CO2 extraction (36.17%). However, the content of sterol in wool oil by supercritical extraction was 294 mg/g, which was 30% and 20% higher than that extracted by n-hexane and petroleum ether, respectively. This also showed that the supercritical CO2 extraction method was more selective than the traditional solvent extraction method, and the target product could be selectively extracted by changing the operating conditions.

2.4 Saponification

Saponification was used to convert fatty acid esters and free fatty acids into fatty acid salts, reducing their solubility in organic solvents, then sterols were extracted by organic solvent through saponification reaction between alkali solution and deodorization distillate [19]. Yan [20] used saponification to extract sterol from soybean oil deodorization distillate and optimized the extraction process. Under the optimized conditions, the yield of sterol was 66.4 mg/g. In recent years, the dry saponification method was also used to make sterols. After the deodorization distillate was saponified at 60~90°C with lime, the solid paste was obtained. Then ethanol was used to leach the paste at low temperature and concentrate. The sterols were obtained. The advantages of dry saponification were simple, safe, non-toxic and solvent saving.

2.5 Microbiological Fermentation

Microbiological fermentation was a new method to make sterol from deodorization distillate. The principle was that the growth process of candida tropicalis could consume fatty acids and glycerides, and hydrolyze sterol ester to release sterol, which could crystallize phytosterol by lowering fermentation temperature. Using this method, Zhao [21] successfully extracted phytosterols from soybean oil deodorization distillates and explored the fermentation process conditions. After microbial fermentation, the sterol concentration was nearly increased by two times. Microbial fermentation provided a new development direction for the industrial production of sterols in the future.

3. THE MACHANISM OF LOWERING BLOOD LIPID OF PHYTOSTEROL

3.1 Reducing cholesterol absorption

Reducing cholesterol absorption was one of the main mechanisms of phytosterol lowering cholesterol in the body. Since plant sterols and cholesterol were both water-insoluble substances, they needed to be emulsified to form micelles in the presence of bile acids to be absorbed by small intestinal cells. Phytosterols were highly similar to cholesterol in molecular structure, thus sterols could enter micelles competitively with cholesterol, thereby reducing the absorption of cholesterol by small intestinal cells [22]. With the in-depth research on the molecular mechanism of cholesterol absorption, the research on the mechanism of plant sterol lowering cholesterol absorption was much more than before.

Cholesterol absorption was a process involving a variety of transporters. The transporter protein (NPC1L1) acted as a crucial role in the absorption of cholesterol in the intestine. NPC1L1 transfers cholesterol molecules transported to the mucosal surface of the small intestine to the cells [23]. For example, after using sitosterol to culture small intestinal epithelial cells for 24h, the mRNA expression of NPC1L1 protein in the cells decreased by nearly 50%, and the expression of NPC1L1 protein also decreased significantly [24]. In addition, the expression of NPC1L1 in liver cells was also significantly decreased after treatment with stigmasterol or sitosterol, suggesting that phytosterol may also reduce the absorption of cholesterol in liver cells [25]. Some studies had shown that ANXA2 / CAV1 compounds also had certain effect in phytosterols reduce cholesterol metabolism activity, ANXA2 and CAV1 and cholesterol ester could form the lipid - protein complexes, making cholesterol ester to be transferred to the cell from the gut, and phytosterol could reduce ANXA2 protein expression, thus reducing intestinal cells on cholesterol absorption. When the mice were fed a high-fat diet containing 2% phytosterol mixture (20% campesterol, 22% stigmasterol, and 41% β -sitosterol), their cholesterol levels were 30% lower than those of the high-fat diet group [26].

3.2 Inhibiting the synthesis of cholesterol

Inhibition of endogenous cholesterol synthesis and secretion was another important mechanism of phytosterol lowering cholesterol in the body. Sterol-regulatory element binding proteins (SREBP) was a cholesterol regulatory element binding protein, which was an intracellular cholesterol sensor located in the endoplasmic reticulum and capable of feedback regulation of intracellular cholesterol. The synthesis of cholesterol was mainly regulated by SREBP. When the intracellular cholesterol was lower than the normal level, SREBP was activated and enters the nucleus, thereby activating the expression of various genes related to the synthesis of cholesterol [27]. The vitro experiments showed that stigmasterol could inhibit the activity of SREBP, thereby reducing the synthesis of endogenous cholesterol [28]. After 28 days of feeding golden hamsters with plant sterol mixture, the SREBP level in the nucleus and the plasma cholesterol content were significantly reduced in the rat liver, indicating that phytosterol may reduce the synthesis of cholesterol by inhibiting the expression of SREBP [29].

The synthesis, decomposition and transformation of endogenous cholesterol were all completed in the liver. The cholesterol-lowering effect of phytosterols was also related to cholesterol metabolism enzymes. 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase was rate-limiting enzyme for cholesterol synthesis, along with other related enzymes such as acetyl CoA carboxylase and malic enzyme. Phytosterol can affect the activity of these rate-limiting enzymes like HMG-CoA reductase. There had been many reports on the effect of phytosterol on the activity of HMG-CoA reductase. Studies had shown that phytosterol can inhibit the activity of HMG-CoA reductase. After feeding rats with stigmasterol (0.5%) for 6 weeks, the activity of HMG-CoA reductase in liver was significantly reduced by 44% and 77%, respectively [30]. The decrease in the activity of HMG-CoA reductase in liver was also observed in rats fed with ergosterol on a high-fat diet [31]. Phytosterols had different effects on expression of HMG-CoA reductase in different tissues. Gavage 50 mg phytosterol ester mixture and 0.25 mg cholesterol in mice at the same time, then detected the expression of genes related to cholesterol synthesis in the intestine and liver at different moment after gavage. The results showed that mRNA expression of small SREBP and its target gene HMG-CoA reductase in mice was significantly up-regulated within 4h after gavage, while mRNA expression of SREBP2 and HMG-CoA reductase in the liver was significantly decreased after gavage for only 15 min [32]. In addition, studies had found that of phytosterols containing C22 double bond, such as stigmasterol, campesterol, ergosterol, can compete with cholesterol precursor chain for activity of sterol C24 sterol reductase (sterol Δ 24 reductase), reducing the conversion of chain sterols to cholesterol, then inhibit cholesterol biosynthesis[33].

IOP Conf. Series: Earth and Environmental Science 615 (2020) 012107 doi:10.1088/1755-1315/615/1/012107

4. CONCLUSION

In this paper, the preparation technology of phytosterol was summarized to provide reference for industrial production of phytosterol and optimization of its process conditions. Then, the mechanism of lowering blood lipid of phytosterol was analyzed to provide reference for the development of functional food and drugs with phytosterol as the active ingredient. In order to study and exploit sterols more comprehensively, the future research direction may start from the following two aspects. First, people had more and more demand for food riches in sterols, which were abundant in the by-products of fat processing. In the process of oil refining, how to ensure the quality of oil and keep the sterol in the oil as much as possible, or how to recover more sterol from the by-products of oil processing are two problems that need to be solved. In addition, the mechanism of lowering blood lipid of phytosterols was relatively complex, which may be the co-existence and synergistic effect of multiple pathways, and the specific mechanism may need to be further studied. In the future, more high-tech methods may be developed to elucidate the mechanism of phytosterol lowering blood lipid.

REFERENCES

- [1] G.P. Moss. 1989. Nomenclature of steroids. IUPAC, Pure Appl Chem 61, 1783–1822.
- [2] Moreau R.A., Nyström M.L., Whitaker B.D., et al. 2018. Phytosterols and their derivatives, Structural diversity, distribution, metabolism, analysis, and health-promoting uses[J]. Progress in Lipid Research, 70, 35-61.
- [3] Robert A.M., Bruce D.W., Kevin B.H. 2002. Phytosterols, phytostanols, and their conjugates in foods: Structural diversity, quantitative analysis, and health-promoting uses[J]. Progress in Lipid Research, 41(6):457-500.
- [4] Blair S.N., Capuzzi D.M., Gottieb S.O., et al. 2000. Incremental reduction of serum total cholesterol and low-density lipoprotein cholesterol with the addition of plant stanol estercontaining spread to statin therapy[J]. The American Journal of Cardiology, 86(1):46-52.
- [5] Grattan B.J. 2013. Plant sterols as anticancer nutrients: evidence for their role in breast cancer [J]. Nutrients, 5(2), 359-387.
- [6] Lin K.M., Koseoglu S.S. 2003. Separation of sterols from deodorizer distillate by crystallization[J]. Journal of Food Lipids, 10(2): 107-127.
- [7] Westvaco Corporation, et al. 2000-05-02. Isolation and purification of sterols from neutrals fraction of tall oil pitch by single decantation crystallization: US, 19980187448[P].
- [8] Kaukas A.B. 1985-04-02. Process for the separation of sterols or mixtures of sterols: Canada, 19820407609[P].
- [9] Wang L. 2011. Study on Extraction Technology of Phytosterol from Pumpkin Seed by Ultrasonic Wave[J]. Journal of the Chinese Cereals & Oils Association, 26(11):62-61.
- [10] Xu Y., Cai S., Song Y., et al. 2013. Optimization of Microwave-Assisted Extraction of Phytosterol from Mulberry Root Bark by Response Surface Methodology[J]. Food Science, 34(18):65-69.
- [11] Hoffmann-la Roche Incorporated. 1996-01-30. Process for tocopherols and sterols from natural sources: US, 19940185571[P].
- [12] Gunawan S., Ju Y.H. 2009. Vegetable oil deodorizer distillate: characterization, utilization and analysis[J]. Separation and Purification Reviews, 38(3): 207-241.
- [13] FACIOLI N.L., Barrera-arellano D. 2002. Optimization of direct acid esterification process of soybean oil deodorizer distillate[J]. Grasas y Aceites, 53(2): 206-212.
- [14] Fu Z., Huang H., Wang H., et al. 2014. The application of new separation technology-molecular distillation regenerated of waste heavy oil[J]. Applied Mechanics and Materials, 5(22): 826-829.
- [15] Ramamurthi S., Mccurdy A.R. 1993. Enzymatic pretreatment of deodorizer distillate for concentration of sterols tocopherols[J]. Journal of the American Oil Chemists' Society, 70:287-295.

IOP Conf. Series: Earth and Environmental Science 615 (2020) 012107 doi:10.1088/1755-1315/615/1/012107

- [16] Comin L.M., Temelli F., Saldaña M.A. 2011. Supercritical CO2 extraction of flax lignans[J]. Journal of the American Oil Chemists' Society, 88(5): 707-715.
- [17] Snyder J.M., King J.W., Taylor S.L., et al. 1999. Concentration of phytosterols for analysis by supercritical fluid extraction[J]. Journal of the American Oil Chemists' Society, 76(6): 717-721.
- [18] Hrabovski N., Sinadinović-fišer S., Nikolovski B., et al. 2012. Phytosterols in pumpkin seed oil extracted by organic solvents and supercritical CO2[J]. European Journal of Lipid Science and Technology, 114(10): 1204-1211.
- [19] Rohr R..2002-02-20. Process for separating unsaponifiable valuable products from raw materials: US, 20020079062 [P].
- [20] Yan F., Yang H., Li J., et al. 2012. Optimization of phytosterols recovery from soybean oil deodorizer distillate[J]. Journal of the American Oil Chemists' Society, 89(7): 1363-1370.
- [21] Zhao G., Hu T., Zhao L. 2014. Fermentation of soybean oil deodorizer distillate with Candida tropicalis to concentrate phytosterols and to produce sterols-rich yeast cells[J]. Journal of Industrial Microbiology and Biotechnology, 41(3): 579-584.
- [22] He W.S., Zhu H., Chen Z.Y. 2018. Plant Sterols: Chemical and Enzymatic Structural Modifications and Effects on Their Cholesterol-Lowering Activity[J]. Journal of Agricultural and Food Chemistry, 66(12): 3047-3062.
- [23] Paalvast Y., De Boer J.F., Groen A.K. 2017. Developments in intestinal cholesterol transport and triglyceride absorption[J]. Current Opinion in Lipidology, 28(3):248-254.
- [24] Jesch E.D., Seo J.M., Carr T.P., et al. 2009. Sitosterol reduces messenger RNA and protein expression levels of Niemann-Pick C1-like 1 in FHs 74 Int cells[J]. Nutrition Research, 29(12):859-866.
- [25] Adkins Y., Kelley D.S. 2010. Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids[J]. Journal of Nutritional Biochemistry, 21(9):781-792.
- [26] Calpe B.L., Joan C.E., Julve J., et al. 2007. Differential intestinal mucosal protein expression in hypercholesterolemic mice fed a phytosterol-enriched diet[J]. Proteomics, 7(15):2659-2666.
- [27] Alphonse P.A.S., Jones P.J.H. 2015. Revisiting Human Cholesterol Synthesis and Absorption: The Reciprocity Paradigm and its Key Regulators[J]. Lipids, 51(5):519-536.
- [28] Yang C., Yu L., Li W., et al. 2004. Disruption of cholesterol homeostasis by plant sterols[J]. Journal of Clinical Investigation, 114(6):813-822.
- [29] Harding S.V., et al. 2010. Hepatic Nuclear Sterol Regulatory Binding Element Protein 2 Abundance Is Decreased and That of ABCG5 Increased in Male Hamsters Fed Plant Sterols [J]. Journal of Nutrition, 109, 120311.
- [30] Batta A.K., Xu G., Honda A., et al. 2006. Stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the rat[J]. Metabolism-clinical & Experimental, 55(3):292-299.
- [31] Moselhy S.S., Kamal I., Kumosani T.A., et al. 2016. Possible inhibition of hydroxy methyl glutaryl CoA reductase activity by nicotinic acid and ergosterol: as targeting for hypocholesterolemic action[J]. African Health Sciences, 16(1), 158-166.
- [32] De Smet E., Mensink R.P., Konings M., et al. 2015. Acute Intake of Plant Stanol Esters Induces Changes in Lipid and Lipoprotein Metabolism-Related Gene Expression in the Liver and Intestines of Mice[J]. Lipids, 50(6): 529-541.
- [33] Carlos, Fernández, Yajaira, et al. 2002. Inhibition of cholesterol biosynthesis by Δ22-unsaturated phytosterols via competitive inhibition of sterol Δ24-reductase in mammalian cells[J]. Biochemical Journal, 366(1):109-19.