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Process for the production of Phytostanols from soybean oil deodorizer distillate

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Abstract

Soybean Oil Deodorizer Distillate (SODD) is obtained as a byproduct of the soybean oil refining process which is rich in sterols, tocopherols, steryl esters, free fatty acids (FFA) and hydrocarbons. The objective of this study was to develop a novel cost effective process for isolation of phytostanols from SODD. Soybean oil Deodorizer Distillate contains approximately 5 to 15 % free sterols of β-sitosterol (35-45 %), campesterol (15-25%), stigmasterol (35-45%) and avenasterol (2-8%). The processing steps for preparation of phytostanols from SODD are briefly as follows. SODD was first dried, mixed with methanol sulphuric acid and esterified. After complete removal of catalyst, the fatty acid methyl esters are distilled out at vacuum with steam supply at 200°C. The residue is then hydrogenated with nickel catalyst and crystallized at 10°C. Crystallized stanols were filtered and washed with chilled hexane to get high purity phytostanols. The major advantage of this process is hydrogenation of phytostanols carried out without a solvent medium. The present study brought out an innovative integrated process for the isolation of phytostanols from SODD. Process simplicity, product quality, less residue and low effluents are the advantages of the study.

Keywords: Phytosterol, Esterification, Soybean oil deodouriser distillate, Phytostanol

Introduction

Recent efforts for the use of industrial waste and byproducts has helped to achieve better and improved methods for recovery and purification of deodorizer distillates. The commercial value of SODD is determined by the content of tocopherols and phytosterols.

Phytosterols are cholesterol homologues found in nuts, fruits, and seeds, and are present in the diet in quantities similar to cholesterol. It is widely accepted that phytosterol intake through food is essential for health (Silbernagel G, 2009). In contrast to cholesterol, phytosterols are poorly absorbed, resulting in circulating concentrations of 1mg/dL (Chan Y M, 2006). The cholesterol-lowering properties of phytosterols have therefore also been exploited in the preparation of functional foods (Brendsel and Green, 2007). Phytosterols cannot be synthesized

by humans, and all plant sterols and stanols in the human body originate from the diet (Jong et al., 2003). They are known to have several bio-active qualities. Their properties for reducing blood cholesterol levels, as well as their other beneficial health effects, have been known for many years (Quilez et al., 2003). Commercial products may be mixtures of phytosterols, phytostanols and their esters. Phytosterols help in regulation of circulating cholesterol through lifestyle changes (Ostlund RE Jr, 2007).

Great deal of interest has been given to the role of phytosterols in the protection from cancer. Raicht et al (1980) suggested that dietary β-sitosterols may offer protection from chemically induced colon cancer. Daily consumption of 1-2 grams of plant sterols or stanols was shown to cause 10-20% reduction in low-density lipoprotein (LDL) cholesterol (Kamal-Eldin and Moazzami, 2009). Stanols are more stable than sterols because of its saturated molecular structure. Stanols are found in small quantities in nature in many plants such as wheat, rye and corn. Plant sterols are a mixture of β-sitosterol, stigmasterol, campesterol and small quantity of other sterols. They are isolated by distillation, extraction, crystallization and washing which results in production of phytosterols. Generally phytostanols are produced after the isolation of phytosterols. They are obtained by hydrogenation of phytosterols using Pd/Ni catalyst in organic solvents (Augustine and Reardon, 1969). In this process, instead of organic solvents, residual fatty acid methyl esters associated with phytosterols was used as a solvent medium for hydrogenation.

Materials and methods

1. Materials

1.1. Chemicals

All chemicals and reagents were of analytical grade. β- sitosterol, campesterol, stigmasterol, and methyl hepta-decanoate standards and lipid standards were obtained from Sigma (St. Louis, MO)

1.2. Raw materials

Soybean Oil deodouriser distillates collected from M/s G-One Agro products, Ahmedabad.

2. Methods

2.1. Physico-chemical parameters

Physicochemical characteristics of SODD and FAME distillate were determined according to standard AOCS methods.

2.2. Fatty acid analysis

Fatty acid methyl esters (FAME) were analyzed using a Hewlett-Packard 5890 series II model gas chromatograph equipped with FID. A DB-23 capillary column (30 m × 0.5 mm × 1 µm; Hewlett-Packard, Avondale, PA) was used for the separation of FAME. Injector and detector port temperatures were 250 and 300°C, respectively. The column temperature was maintained at 100°C for 1 min and increased to 180°C at the rate of 5°C/min, then maintained at that temperature for 15 min. Nitrogen at a flow rate of 20 mL/min was used as a carrier gas with air flow at the rate of 400 mL/min and hydrogen 40 mL/min. FA were identified using standards. The amount of lipid sample was determined by relating the total area of the FAME peak.

2.3 High-performance liquid chromatography (HPLC)

Sterols determined by using an HPLC system equipped with an LC-10AD pump (Type 7125; Rheodyne, Cotati, CA), 20-µL sample loop, UV-vis detector (Shimadzu Corporation, Kyoto, Japan) Sterols composition of DODs were determined according to the HPLC method of Holen (1985). A Zorbax C18 reversed-phase column (4 mm i.d. × 25 cm length; Agilent Technologies, Palo Alto, CA) filled with 5-µm particles was used for the analysis. The

solvent system MeOH/H_2O in a ratio of 96.5:3.5 (vol/vol) at a flow rate of1.2 mL/min was used for the separation of sterol isomers. Peaks were detected at 206 nm. Calibrations of standard curves were done by using standard sterol solutions in the range of 1–20 µg/20 µL**.**

2.4. Process steps for the production of phytostanols

2.4.1. Drying

Preconditioning of the distillate for esterification is an essential process for phytostanol processing. Therefore initial moisture content should be reduced to the limit by vacuum drying 85-90^oC. This drying process was carried out in a vessel with controlled heating, stirring and vacuum provision.

2.4.2. Esterification free fatty acid and neutral lipids

The dried DOD was taken in the reaction vessel and 2% methanolic sulphuric acid 1:1 ratio was added and heated to a temperature of 65^oC for 4 hours in a constant temperature water bath.

2.4.3. Removal of excess methanol

The excess methanol used in this reaction mixture was distilled out using laboratory model distillation set up at 75 ^{o} C.

2.4.4. Water washings to remove acid catalyst

This is one of the most important steps in this process. The acid catalyst was removed by hot water washing. The pH of the washed water was checked until it is neutral.

2.4.5. Separation of fatty acid methyl esters (Bio diesel)

Fatty acid methyl esters separated in a reaction set up contains heating, steam stripping, condenser and vacuum. Fig 1 shows the distillation set up used for the recovery of FAME. The FAMEs were separated by distillation at 200^oC with steam stripping.

2.4.6. Hydrogenation of Phytostanols

The residue after the distillation of FAME contains phytosterols which were converted to phytostanols by hydrogenation. 500 g sample filled in a 1L stainless steel hydrogenation vessel, with 10 g nickel catalyst. The air inside the reaction vessel was removed by vacuum and hydrogen gas under pressure was maintained at 5 kg/cm^2 and heated up to 180 ^oC using external heating coil. The reaction mixture was agitated by shaking and continued the reaction for 4 hrs.

2.4.7. Crystallization of Phytostanols

Phytostanols was separated from the mother liquor by crystallization. The hydrogenated mass was collected and kept in a crystallizer and maintained the temperature at 10^oC in a cold room overnight.

2.4.8. Filtration

Crystallized stanols from the mother liquor was separated by filtration using 1 liter size buckner funnel with filter cloth.

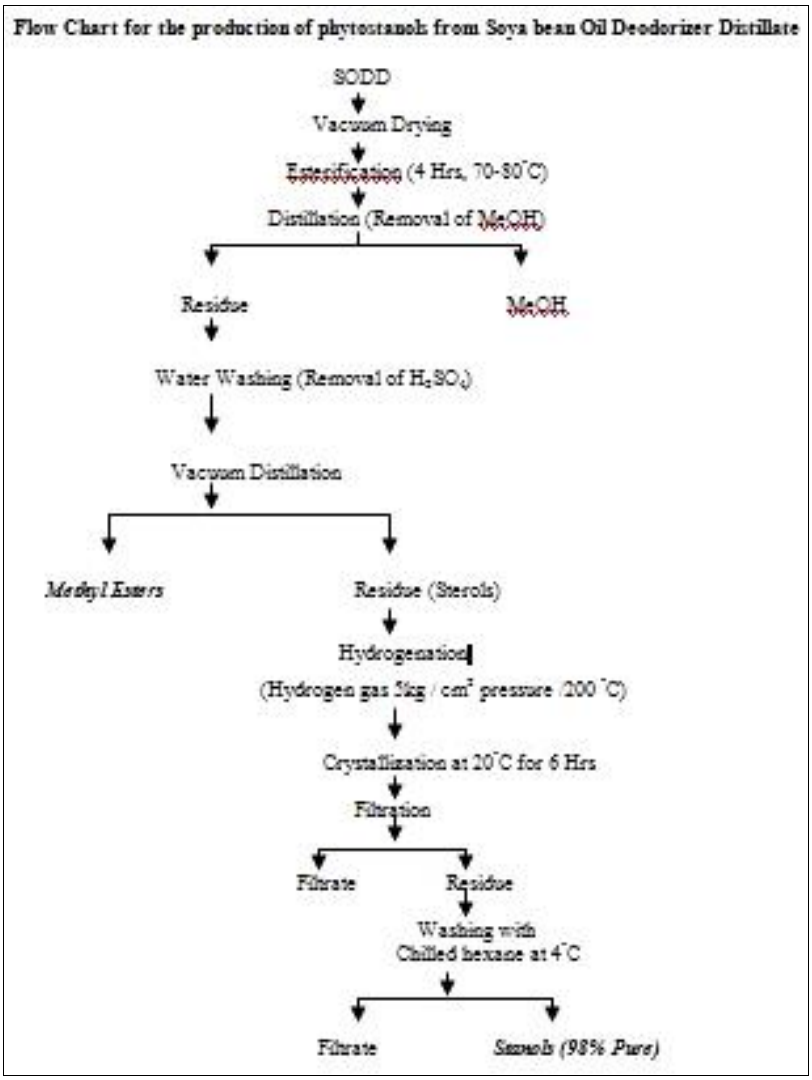
2.4.9. Hexane washing

During filtration of crystallized stanols, a small amount methyl esters, tocopherols etc will retain along with the filter cake as impurity. This can be removed by washing with chilled hexane at 5-10^oC.

2.5. Spectroscopic studies

The mass spectra (MS) of purified phytostanols were recorded on Agilent Technologies 1200 series Mass Spectrometer (Chem station software).

Infrared (IR) spectrums of the stanols were recorded on Perkin-Elmer spectrum 100-FTIR instrument (UK).



Results and discussion

1. Chemical composition of SODD

Physicochemical characteristics of SODD are given in Table.1. The proximate analysis shows that it contains mainly 25.55% unsaponifiable matter. This unsaponifiable matter is the source of many bioactive compounds.

Table 1: Chemical composition of SODD.

Sl.No.	Sample	SODD %
1.	Moisture	2.51
2.	FFA	34.43
3.	Neutral lipids (calculated)	36.15
4.	Unsap matter	25.55
5.	Total sterol content	9.46
6.	Tocopherols	7.88

2. Phytosterol composition of SODD

The phytosterol composition of SODD is given in table.2. β- Sitosterols are rich in SODD and the total sterol content is 9.45 %.

Table. 2. Total sterol content and its composition by HPLC method.

Sample	Sterols				Total (%)
	β-Sitosterols (%)	Campesterols (%)	Stigmasterols (%)	Other sterols (%)	
SODD	3.77	2.21	2.59	0.88	9.45

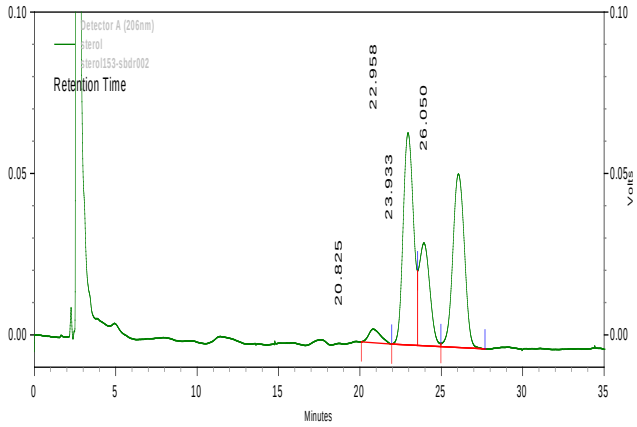


Fig.1 HPLC chromatogram for sterols from SODD

3. Fatty acid profile of SODD

The fatty acid composition of the methyl esters was estimated using GC. The fatty acid profile is given in Table 3. The fatty acid profile of the SODD distillate is similar to soybean oil except that palmitic acid is very high and linolenic acid is less than pure soybean oil.

4. Process steps involved in isolation of phytosterols

4.1. Drying

The moisture content of SODD was 2.51% (Table.1). Moisture content is one of the major parameter in process conditions as more than 0.05% moisture will affect methylation. After drying the SODD moisture content was reduced to 0.02%.

4.2. Esterification of SODD

After 4 hours, the completion of esterification was monitored using TLC. At the end of the reaction, neutral lipids and fatty acids are converted to their corresponding methyl esters. Analytical results of the products were given in Table.3 and 4.

Table 3: Fatty acid Composition of the methyl ester distillate of SODD (Biodiesel).

Sl.No	Name of Fatty acid	Relative %
1	Myristic acid(C14)	3.06
2	Palmitic acid (C16)	52.91
3	Stearic acid(C18)	2.65
4	Oleic acid (C18:1)	18.90
5	Linoleic acid (C18:2)	21.15
6	Linolenic acid (C18:3)	1.32

Table 4: Chemical composition of fatty acid methyl ester distillate of SODD (Biodiesel).

Characterstics	**Composition**
Acid Value	0.65 %
Unsaponifiable matters	2.5 %
Iodine Value	106
Free glycerol	0.01 %

4.3. Removal of methanol

After the completion of esterification, 80 – 90 % methanol remains as excess this methanol was separated by distillation at 60 – 65°C and reused.

4.4. Water washing

In this process step, hot water was added to the esterified mass to facilitate the separation of acid catalyst and residual methanol. The major problem associated with acid catalyzed esterification reactions is catalyst removal. In most cases

neutralization of acid catalyst with alkali is followed. Here, acid catalyst is removed by water washing at 80°C.

4.5. Separation of Fatty Acid Methyl Esters (FAME)

The distlled FAME was light yellow in colour and the physico chemical properties are given in table 3&4.

4.6. Hydrogenation

After hydrogenation, total unsaturated fatty acids and phytostanols are converted into saturated fatty acids and phytostanols.

4.7. Crystallization of phytostanols

The residual methyl esters and other component present in hydrogenated mixture served as a crystallization medium that facilitates crystallization of stanols. At 10°C stanols were crystallized and settled in the crystallization vessel.

4.8. Filtration

The crystallized distillate was semisolid in nature which makes filtration difficult. Under vacuum the fatty acid methyl esters and crystallized phytostanols are separated. The filtrate contains methyl esters, sterylesters, tocopherols, squalene etc.

4.9. Hexane washing

Phytostanols have less solubility in hexane at normal conditions; solubility is again reduced at lower temperatures. Therefore chilled hexane was used for the purification of stanol crystals. Chilled hexane at 5 °C was passed through the crystal bed. The impurities were dissolved in chilled hexane and pure white stanol crystals are obtained.

5. Spectroscopic studies

The molecular ion peak of two major stanols was detected in mass spectrum. β-Sitostanol at 413 m/z and stigma stanols at 415 m/z (Fig.2)

FTIR spectrum of the purified phytostanols from SODD

IR spectrum of both standard stanol and sample stanol are similar and both spectrum shows peak in the range of 3640-3200 cm^{-1} which is the characteristic peak of OH stretching vibrations in alcohols. Comparisons of FT-IR Spectra of sterols and stanols shows that sterol has a peak at 1000-650 cm^{-1} region corresponds to C==C stretching frequency, but stanol does not shows peaks in this region because of its lack of unsaturation (Fig.3).

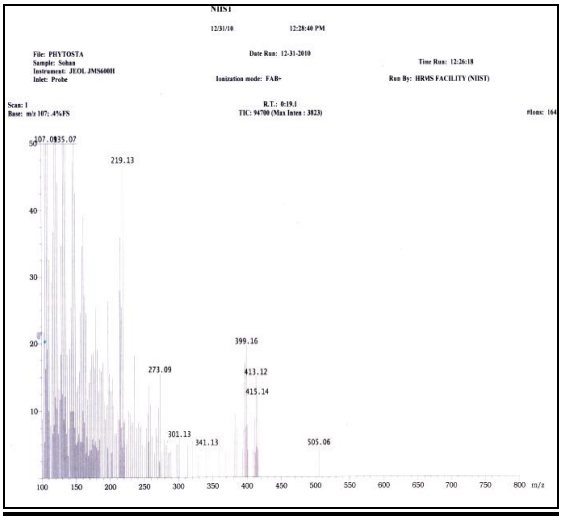


Figure 2: Mass spectrum of the purified phytostanols from SODD.

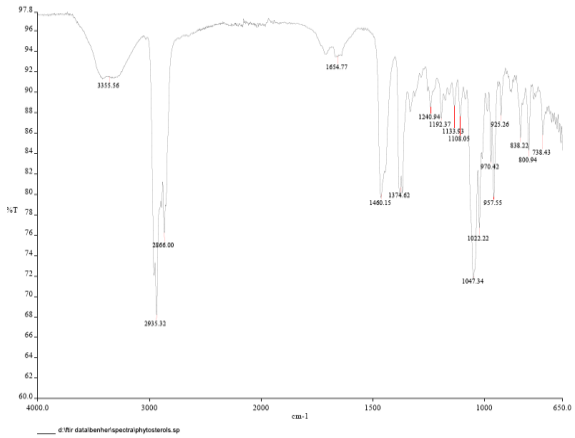


Figure 3: FTIR spectrum of the purified phytostanols from SODD.

Conclusion

The present study developed a cost effective process technology for production of phyto stanols from soya bean oil deodorizer distillates.

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References

AOCS., 1997. Official Methods and Recommended Practices of the AOCS, 5th edn., edited by D. Firestone, AOCS Press, Champaign. Methods Ca 12-55, Ca 5a-40, Cc 13e-92, Cd 3-25, Cd 1-25, Cc 10a-25, Ca 2d-25, Cc 6-25, Cc 7-25, Ca 6a-40.

Augustine, R.L., Reardon Jr, E.J., 1969. The palladium catalyzed hydrogenation of cholesterol, Org. Prep. Proced. Int., 1, 107-109.

Brendsel, J., Green, S.J., 2007. Regarding the potential perils of phytosterols. Atherosclerosis. 192(1), 227-9.

Cantrill, R., 2008. Phytosterols, Phytostanols and their Esters: Chemical and Technical Assessment (CTA) [Prepared by Richard Cantrill, Ph.D., reviewed by Yoko Kawamura, Ph.D., for the 69th JECFA]. Food and Agriculture Organization of the United Nations (FAO), Italy.

Chan, Y.M., Varady, K. A., Lin, Y., Trautwein, E., Mensink, R. P., Plat, J. and Jones, P. J.H., 2006. Plasma Co,ncentrations of Plant Sterols: Physiology and Relationship with Coronary Heart Disease. Nutrition Reviews, 64, 385–402.

de Jong, A., Plat, J., Mensink R.P., 2003. Metabolic effects of plant sterols and stanols. J Nutr Biochem 14(7), 362-9.

Holen, B., 1985. Rapid Separation of Free Sterols by Reversed-Phase High Performance Liquid Chromatography, J. Am. Oil Chem.Soc 62, 1344–1346.

Kamal-Eldin, A., Moazzami, A., 2009. Plant sterols and stanols as cholesterol-lowering ingredients in functional foods Recent Pat Food Nutr Agric. 1(1), 1-14.

Ostlund, R.E. Jr., 2007. Phytosterols, cholesterol absorption and healthy diets.Lipids. 42(1), 41-5.

Quilez, J., GarcIa-Lorda, P, Salas-Salvadó., 2003. Potential uses and benefits of phytosterols in diet: present situation and future directions. J. Clin Nutr. 22(4), 343-51.

Raicht, R.F., Cohen, B.I., Fazzini, E.P., Sarwal, A.N., Takahashi, M., 1980. Protective effect of plant sterols against chemically induced colon tumors in rats Cancer Res. 40(2), 403-5.

Silbernagel G., Fauler, G., Renner, W., Landl, E. M., Hoffmann, M. M., Winkelmann, B. R., Boehm, B. O., März, W., 2009. The relationships of cholesterol metabolism and plasma plant sterols with the severity of coronary artery disease. J. Lipid Res. 50, 334–341.