Chemical Science Review and Letters ISSN 2278-6783
Research Article

Synthesis of Triacontanol Concentrate from Crude Rice Bran Wax by Microwave Treatment and Molecular Distillation

Sumit Nandi*, Sarbani Ganguly and Rupa Bhattachryya

Department of Chemistry, Narula Institute of Technology, Agarpara, Kolkata 700109, West Bengal, India,

Abstract

Triacontanol concentrate is prepared from crude rice bran wax (RBW). RBW is a byproduct of rice bran oil industry and has been recognized as a potential source for the synthesis of policosanols which consist mainly of triacontanol, octacosanol, dotriacontanol and hexacosanol. In the present research investigation, a detailed study has been made for the extraction and purification of triacontanol concentrate from crude RBW by microwave digestion and molecular distillation (MD) method. Triacontanol acts as a plant growth regulator and is a powerful inhibitor of lipid per oxidation. It may exert anti inflammatory effect and has the capacity to lower low-density lipoprotein and raise high-density lipoprotein cholesterol. In our study, the operating conditions for the hydrolysis of deoiled and bleached RBW in microwave oven are as follows: 1300 watt, 230 V-50 Hz, 1:2 of wax of 6% potassium hydroxide (w:v) and 35 minutes at microwave temperature. After hydrolysis, the extracted policosanols containing 42.95% triacontanol is purified by MD and the optimum conditions are temperature 153^0C and pressure 62.37 Pascal. This treatment yields triacontanol concentrate containing 74.18% triacontanol along with 15.83% octacosanol, 5.23% dotriacontanol, 3.19% hexacosanol and 1.28% tetracosanol at residue detected by high performance liquid chromatography (HPLC) method.

Correspondence
Sumit Nandi,
Email: sumitnandi5@gmail.com
Ph.-09830548335/09804469759.

Keywords: Microwave, Policosanols, Triacontanol, Rice bran wax, Molecular distillation.

Introduction

Rice bran wax (RBW) is an important by product of rice bran oil industry. It is obtained during refining of rice bran oil obtained from rice bran (source: *Oryza sativa* -Family Graminae). RBW is a mixture of long chain fatty alcohols and composition varies depending on the source, processing methods and conditions. RBW is edible and can serve as a substitute for carnauba wax due to its high melting point. This wax can be upgraded by deoiling for use in food and pharmaceutical industries. RBW which is mostly sold at very cheap price can be processed to produce light coloured de-oiled wax of high industrial value. Some of the commercial applications of wax are as an enteric coating for candies and lozenges, for preparation of wax emulsion, as a plasticizing material in chewing gums and also as an ingredient in manufacture of carbon papers.

RBW can be converted into long chain fatty alcohols like policosanols. The composition of policosanols is about 90% aliphatic alcohols [1] but varies depending on the source of material and the method of extraction. Recent studies show that policosanol accounts 95% or so, even numbered aliphatic alcohols where triacontanol (C30) is the predominant component. Others are octacosanol (C28), dotriacontanol (C32), tetracosanol (C24) and hexacosanol (C26). Long chain fatty alcohols have been reported to lower plasma cholesterol in humans. Policosanols have both nutritional and pharmaceutical applications. Policosanols have been suggested as a potential therapy for use in slowing the progression of Alzheimer's disease. It can also be used as anticoagulant, antiplatelet and anti diabetic agents.

The origin of research on cholesterol-lowering effects of policosanol dates back to 1972. In several double-blind, randomized, placebo-controlled clinical trials, as well as animal studies, sugar cane policosanol has been clinically proved to reduce total cholesterol, strongly reduces LDL cholesterol and raise the level of HDL

cholesterol. Outcomes of over 60 clinical trials have been reviewed in detail and reports of cholesterol lowering are very consistent [2]. Cholesterol lowering effect of octacosanol was confirmed when sugarcane rinds or wax extracted from sugarcane was found to lower cholesterol in rats [3, 4]. The effect was also noted when wax was added to the diet of rats and purification of the active principles led to the identification of long chain alcohols as cholesterol-lowering agents. Reports suggest that 5-20 mg per day of mixed C_{24}-C_{34} alcohols, including octacosanol and triacontanol, lower low-density lipoprotein (LDL) cholesterol by 21-29% and raise high-density lipoprotein cholesterol by 8-15%. More recently, it has been reported that octacosanol and policosanol improve reaction time, which possibly indicated a neurological effects [5].

Triacontanol has also been identified as a plant growth regulator, found in the plant cuticle waxes and in beeswax as the palmitate ester [6]. Its efficiency has been proved for field crops like barely, rice, tomatoes, maize, lettuce, cucumber, potatoes, cauliflower, brinjal, chilies, etc. Recent [7] research has shown that triacontanol is a powerful inhibitor of lipid per oxidation and it may exert anti inflammatory effect. Triacontanol is effective on biosynthesis of secondary metabolites and in plant, it regulates physiological and biochemical process.

Triacontanol has been isolated by many methods but no such method is simple and cost effective. Cravotto et. al. [8] extracted policosanols from rice bran wax by high intensity ultrasound and they claimed that sonochemical conditions are effective for yielding policosanols. Recently Jaybhay et al [9] isolated crude triacontanol from rice bran wax by saponification and extraction method and they obtained the product as 13.3% pure. Chen et al [10] used response surface technology for the purification of crude octacosanol extract from rice bran wax by molecular distillation.

In our study, we use microwave digestion method along with molecular distillation for preparation of triacontanol extract from crude RBW. Here, crude RBW was first defatted by isopropanol fractionation, double bleached by hydrogen peroxide and benzyl peroxide to obtain a light colour hard wax. The deoiled and bleached wax was then hydrolyzed in a microwave oven for about 35 minutes in presence of alkali to produce policosanols. Policosanols are then treated in molecular distillation to produce triacontanol concentrate maintaining certain temperature and pressure and final product compositions have been identified by HPLC technique.

Materials and Methods

Materials

Crude RBW was obtained from Sethia Oil Mill, Burdwan, West Bengal, India. Isopropyl alcohol was purchased from E. Merck (India) Pvt. Ltd. Other solvents and chemicals used were of analytical grade.

Purification of crude RBW

The purification process of crude RBW involves two stages. First stage was deoiling of crude RBW which was done by melting sample. Melted RBW was mixed in isopropyl alcohol (1:3, w:v) and refluxed at 80^0C for 50 min. After that, the mixture was cooled and deoiled wax was centrifuged. Second stage involved double bleaching of deoiled wax. For this purpose, deoiled wax was taken in a 1000mL conical flask and refluxed with 100 mL hydrogen peroxide (5%) and 200 mL deionised water for 1.5 h at 95^0C. After that, 100 mL benzoyl peroxide (5%) was added and again refluxed for 1 h at 95^0C. The material was then cooled and centrifuged. The bleached wax crystals was then washed with deionised water and dried.

Hydrolysis of deoiled and bleached RBW in microwave oven

For microwave treatment of deoiled and bleached RBW, microwave oven used was manufactured by LG company, Korea. Model name was MS-257 PL, Intellowave (1300 watt, 230 V-50 Hz). RBW and KOH solution (1:2 w:v of wax and 6% potassium hydroxide) were taken in a specially designed conical flask with air condenser. The mixture was then digested in microwave oven for about 35 minutes. The hydrolysed wax was cooled and washed with hot water several times for removing excess KOH and soap formed during the process. The residual fatty alcohol part (policosanols) was isolated by centrifugation and dried.

Purification of triacontanol concentrates by MD

Triacontanol concentrate was prepared from policosanols using the apparatus MD. The MD apparatus (Rotating film type) with the capacity of 2000 mL (SIBATA Scientific Co. Ltd., Japan, Model no MS-300) was a falling film type apparatus and provided with a rotating wiper that continuously rubs the falling film on the evaporating surface.

Policosanols were taken in the MD flask after being heated and melted. Then the vacuum pump was turned on and temperature was raised. When the vacuum pressure was steady and reached at 62.37 Pascal and the temperature of the feed was at 153^0C, the bottom valve of the flask was opened. The heated feed was immediately flowed down to the evaporating surface and the rotating wiper was started to rubbing on the evaporating surface. More volatile components were separated and concentrated in the condensing flask due to high temperature and high vacuum and became the distillate. Less volatile components flowed down along the evaporating surface which was ultimately collected at the bottom flask as residue (triacontanol concentrate).The feed rate to the evaporating surface was maintained at 2 ml/minute.

Analysis of triacontanol concentrate by HPLC

Fatty alcohols were identified by the instrument HPLC of Waters (I) Pvt. Ltd. The HPLC instrument was provided with Binary HPLC Pump 1525 and Waters Dual Absorbance UV Detector 2487 and Refractive Index Detector 2414. The HPLC column was Novapak bonded C18 having micro particulate silica of particle size of about 5 μm. The isocratic flow rate was 0.5ml/min. The whole system was supported by Breeze 2000 software.

For detection of fatty alcohols, the mobile phase consisted of HPLC grade hexane, acetonitrile and isopropyl alcohol in the ratio of 75:15:10. Here refractive index detector was used. 10 μl of the solution of the sample was injected and the fatty alcohols were detected according to their retention time and quantified with reference to the standard sample.

Results and discussions

Upgradation of Crude Rice Bran Wax

Crude RBW is dark brown in colour and the oil content of the crude wax varies from 20 to 85% depending on the separation and processing conditions. Table 1 lists the characteristics of crude RBW. It contains about 50.56± 0.434% oil with slip melting point 68-69°C. Crude wax has P-content 0.078± 0.002%, acid value 13.5± 0.134, saponification value 112.5± 0.734, unsaponifiable matter 41.2± 0.364 and iodine value 23.3± 0.195. Acid value, iodine value, saponification value, phosphorous content and unsaponification value were determined according to standard method described in the official and tentative methods of American Oil Chemists' Society (2002).

Table 1 Characteristics of crude rice bran wax

Properties	Values (%)
Moisture	2.8± 0.023
Oil content	50.56± 0.434
Acid value	13.5± 0.134
Phosphorous content	0.078± 0.002
Saponification value	112.5± 0.734
Unsaponification Value	41.2± 0.364
Iodine value	23.3± 0.195
Slip melting point	68-69^{0}C

Values are reported as mean ± s.d., where n=3 (n=no of observations).

For the up gradation purpose, crude RBW was deoiled and bleached. It was not degummed as it was previously degummed. Deoiling of wax is an important step as many factors like reflux temperature, solvent selection, solvent to wax ratio are involved. In our study, the optimal conditions are wax: isopropyl alcohol is 1:3 (w:v), refluxed

temperature maintained at 80^0C for 50 min. After that the mixture was cooled and during cooling proper care must be taken as fast cooling produces small and uneven sizes of crystals which would affect the separation of oil from wax effectively. Deoiled wax was then double bleached with hydrogen per oxide (5%) and benzoyl peroxide (5%) to prepare light coloured wax. After bleaching, the light coloured wax was separated by filtration using a Buchner funnel. The wax cake was then washed and dried under vacuum at 20^0C.

Deoiling with IPA of crude RBW reduces oil content <1%. **Table 2** shows the characteristics of deoiled and bleached RBW. After removing oil, the slip melting point of wax shows 80-81ºC. The acid value of deoiled wax reduced to 1.14± 0.013. The saponification value, unsaponification value and iodine value of deoiled wax were 76.5± 0.054, 62.2± 0.039 and 9.7± 0.004 respectively.

Table 2 Characteristics of deoiled and bleached rice bran wax

Properties	Values (%)
Moisture	0.01
Oil content	< 1
Acid value	1.14± 0.013
Phosphorous content	0.098± 0.001
Saponification value	76.5± 0.054
Unsaponification Value	62.2± 0.039
Iodine value	9.7± 0.004
Slip melting point	80-81^{0}C

Values are reported as mean ± s.d., where n=3 (n=no of observations).

Hydrolysis of RBW

A specially designed conical flask fitted with an air condenser was used in microwave oven to hydrolyse the deoiled and light coloured wax. Advantage of microwave heating is that it does not heat the flask, only the desired material is heated. Desired material absorbs energy from the microwave in a process called electromagnetism, a phenomenon associated with electric and magnetic fields and their interactions with each other. After digestion, the digested wax was washed with boiling water for several times. Hot water wash was essential to remove excess KOH and the soap formed during microwave digestion. After washing and vacuum drying, the product obtained was enriched with triacontanol.

Fig. 1 indicates the hydrolysis of deoiled and bleached RBW through conventional hydrolysis as well as microwave digestion. For the same time duration, microwave hydrolysis gives excellent result compared to conventional procedure. It can be inferred from **Fig. 1** that for the same time duration microwave treatment gives 36% free fatty acids whereas conventional hydrolysis produces only 3.5% free fatty acid. So, it can be stated that the rate of chemical hydrolysis of deoiled wax ester under microwave is very fast compared to conventional chemical hydrolysis. Table 3 shows the composition of microwave digested HPLC analysed policosanols.

Isolation of triacontanol concentrates by MD

The purification of triacontanol concentrate was conducted at 62.37 Pascal maintaining temperature 153^0C. Isolation of triacontanol extract from policosanols is carried out in MD apparatus based on the knowledge of mean free path of molecules. With increasing temperature, vapour pressure quickly increases. As a result, mean free path of the molecule becomes longer causing higher rate of collisions among the molecules in the gap between the evaporator and the condenser. Therefore, more volatile components due to high temperature and high vacuum

became the distillate and less volatile components was collected at the bottom flask as residue. So, heavier fractions (triacontanol concentrate) was the predominant one in the condenser compared to other fractions.

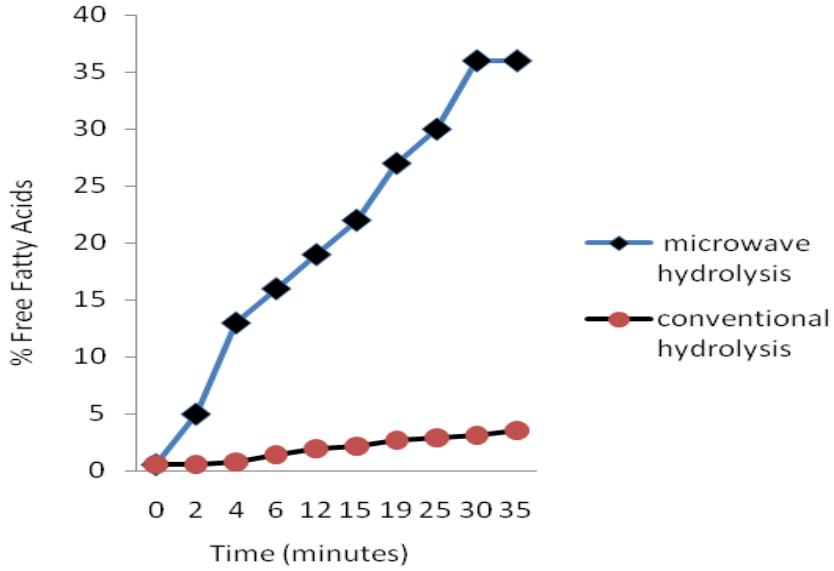

Fig.1: Comparison between microwave hydrolysis and conventional hydrolysis

Table 3 Compositions of policosanols after microwave digestion

Compositions	Values (%)
Tetracosanol	5.86 ± 0.28
Hexacosanol	11.90 ± 0.3
Octacosanol	25.24 ± 0.78
Triacontanol	42.95 ± 0.66
Dotriacontanol	14.02 ± 0.27

Values are reported as mean ± s.d., where n=3 (n=no of observations).

Table 3 shows that hydrolysed wax contains 42.9±0.665% triacontanol followed by 25.24± 0.78% octacosanol, 14.02± 0.27% dotriacontanol, 5.86± 0.28% tetracosanol and 11.9± 0.3% hexacosanol.

HPLC analysis of triacontanol concentrate

HPLC analysis is quite helpful for identifying the fatty alcohols present in triacontanol concentrate. Analysis shows that (**Table 4**) residue (triacontanol concentrate) from molecular distillation contains 74.18± 0.85% triacontanol while octacosanol, dotriacontanol, hexacosanol and tetracosanol are 15.83±0.49%, 5.23±0.39%, 3.19±0.21% and 1.28± 0.1% respectively. In comparison, more volatile product (distillate) comprises of 33.8±0.46% triacontanol, 39.3± 0.72% octacosanol, 12.5± 0.33% hexacosanol, 8.3±0.31% dotriacontanol and 6.1±0.31% tetracosanol. All analysis was done by comparing the HPLC pick of standard sample.

Conclusion

The present study investigated the preparation of triacontanol extract from crude rice bran wax. The concept of utilizing microwave heating to hydrolyze rice bran wax followed by molecular distillation for the production of triacontanol concentrate is an important development. Microwave digestion is a simple and new technique for the hydrolysis of deoiled and bleached wax. The results indicated that molecular distillation of the hydrolysed wax was

the effective purification method for triacontanol concentrate. Distilling temperature and pressure play significant role for this process. So identification of these parameters is very important in purification process. However, identification of parameters not only depends on the absolute amount of triacontanol molecules in the residue but also by the relative amount of other components that could reach the residue. Maintaining temperature at 157^0C and pressure 62.37 Pascal in MD, 74.18% triacontanol in the residue has been obtained along with other policosanols. Distillate may be the useful source for the production of octacosanol extract as this fraction contains 39.3% octacosanol along with 33.8% triacontanol and other higher alcohols. Finally it can be said that more sustained study would be appreciated for the future researchers to optimize the process in order that it may find importance in industrial scale also.

Table 4 Comparisons of compositions of policosanols

Components	Before MD	After MD	
	Hydrolysed wax (%)	Distillate (%)	Residue (%) (Triacontanol concentrate)
Tetracosanol	5.86 ± 0.28	6.1 ± 0.31	1.28 ± 0.1
Hexacosanol	11.90 ± 0.3	12.5 ± 0.33	3.19 ± 0.21
Octacosanol	25.24 ± 0.78	39.3 ± 0.72	15.83 ± 0.49
Triacontanol	42.95 ± 0.66	33.8 ± 0.46	74.18 ± 0.85
Dotriacontanol	14.02 ± 0.27	8.3 ± 0.31	5.23 ± 0.39

Values are reported as mean ± s.d., where n=3 (n=no of observations).

References

[1] R. Mas, *Journal Nutritional Science and Vitaminology*, **2000**, 25, 569-586.

[2] I. Gouni, H. K. Berthold, *American Heart Journal*, **2002**, 143, 356-365.

[3] H. Sho, I. Chinen, K. Uchihara, N. Fukuda, *Journal Nutritional Science and Vitaminology*, **1981**, 27, 463-470.

[4] H. Sho, I. Chinen, N. Fukuda, *Journal Nutritional Science and Vitaminology*, **1984**, 30, 553-559.

[5] G. Fontani, D. L. Maffei, *Neuropsychobiology*, **2000**, 41, 158-165.

[6] R. R. Iyer, V. R. Mamdapur, *Indian Journal of Chemistry*, **1986**, 25B, 1216-1219.

[7] X. Chen, H. Yuan, R. Chen, L. Zhu, B. Du, Q. Weng, G. He, *Plant Cell physiology*, **2002**, 43(8), 869-876.

[8] G. Cravotto, A. Binello, G. Merizzi, M. Avogadro, *European Journal of lipid Science and Technology*, **2004**, 106(3), 147-151.

[9] S. Jaybhay, P. Chate, A. Ade, *Journal of Experimental Sciences*, **2010**, 1(2), 26.

[10] F. Chen, T. Cai, G. Zhao, X. Liao, L. Guo, X. Hu, *Journal of Food Engineering*, **2005**, 70, 47-53.

Publication History
Received 28th Sep 2013
Revised 10th Oct 2013
Accepted 20th Oct 2013
Online 05th Jan 2014