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# Functional Foods from Soybean Oil Deodoriser Distillate Using *Candida Antarctica*

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**Abstract:** Soybean oil deodoriser distillate (SBO DD) is an important by product of vegetable oil refining industries. SBO DD can be utilized for the synthesis of novel functional food ingredients which contains triacylglycerols (TAGs), diacylglycerols (DAGs) and monoacylglycerols (MAGs) along with sterols, tocopherols and hydrocarbons. In the present study, fatty acids present in SBODD were esterified with glycerol of varying amounts (3:1, 3:1.1, 3:1.2 and 3:1.4 of DD: glycerol molar ratio) for 8 hrs in the presence of non specific enzyme NS 40013 (*Candida Antarctica*) at  $63 \pm 2^\circ\text{C}$ . After completion of esterification reaction, the product mixture containing significant amount of TAGs, DAGs and MAGs were purified by molecular distillation to remove excess of free fatty acids and other non desirable components. The final glycerides rich in sterols, tocopherols and hydrocarbons (HC) can be utilised in various food formulations.

**Keywords:** Deodoriser distillate, *Candida Antarctica*, Tocopherols, Sterols, Acylglycerols.

## I. INTRODUCTION

Functional foods refer to foods and their components that provide a health benefit beyond basic nutrition due to certain physiologically active components. They can play a vital role in reducing the risk of disease and promoting good health. Biologically active components in functional foods impart health benefits or desirable physiological effects [1, 2]. Rapid advances in food science and technology, an aging population, the rapid rise in health care costs, and changing food consumption, functional foods play a significant role for the last few decades throughout the world which ultimately create a large impact on the functional foods market [3].

Deodorizer distillate is an excellent source in this respect for the valuable compounds such as phytosterols, tocopherols, sterols and hydrocarbons (mainly squalene), which can be recovered and used as food additives [4,5,6,7]. Their commercial value however, is mainly dependent on their tocopherol content [8]. Different deodoriser distillates or fatty acid distillates are used for this purpose like soybean, canola and sunflower [9,10], palm [11,12,13], rice bran oil [14] etc. SBODD, one of the important by products of vegetable oil refining industry, has been considered as an important source for the preparation of functional foods as it contains sterols, tocopherols and hydrocarbons. Different process technology has been adopted for the preparation of functional foods from SBODD by different researchers. Facioli and Barrera-Arellano used chemical [15] as well as enzymatic method [16] using SBODD for this purpose. In another study, Gunawan, Kasim and Ju [17] separated and purified squalene from SBODD. They also identified steroidal hydrocarbons from SBODD by suitable process technology [18]. Khatoon, Raja Rajan and Gopala Krishna [19] made a detailed study about physicochemical characteristics and composition of Indian SBODD and also recovered phytosterols. Nagao et al. [20] tried to improve purification technology of sterols and tocopherols from SBODD. But functional foods from SBODD with a composition of neutral glycerides and sterols, tocopherols and hydrocarbons have little been studied so far. So in the present study, a bioprocess technology has been studied to produce neutral glycerides along with micronutrients using non specific enzyme *Candida antarctica* maintaining a temperature of  $63 \pm 2^\circ\text{C}$  for 8 hrs. The experimental study contributed good quality bioesterified and purified products which may be utilized as functional foods.

## II. EXPERIMENTAL

### A. Materials

SBODD was collected from M/s. Sethia Oil Mills, Burdwan, West Bengal, India. The non-specific immobilized lipase NS 40013 (*Candida Antarctica*) was a kind gift of Novozyme South Asia Pvt. Ltd. Bangalore, India with ester synthesis activity of 10000 Propyl Laurate unit/g (PLU/g). Glycerol (A.R.) were purchased from E.Merck (India) Pvt. Ltd. Except otherwise specified all other chemicals used were A.R. Grade.

### B. Bleaching of SBODD

About 500 gm SBODD was taken in 1L round bottom flask and placed on a boiling water bath with shaking for 30 minutes under vacuum (2-4 mm Hg pressure). Then 4% Tonsil earth and 0.5% activated charcoal were added and shaken vigorously for 20 minutes under vacuum. After that, the whole material was cooled, filtered under vacuum and the bleached SBODD was stored in a refrigerator at -20°C for further study.

### C. Enzymatic esterification of bleached SBODD

The bleached SBODD and glycerol were taken in a round bottom flask for esterification purpose and stirred by a magnetic stirrer at 65±2 °C under reduced pressure for 8 hrs in the presence of 5% enzyme. The glycerol was used in different proportions based on the amount of SBODD. The progress of esterification reaction was observed through the estimation of free fatty acids of the reaction mixture. After 8 hrs of reaction, the product mixture was isolated for purification after removal of enzyme through filtration.

### D. Purification of product

The product was purified in a molecular distillation unit (SIBATA Scientific Co. Ltd., Japan, Model – MS 300) at 153-155°C temperature and 18-20 Pascal pressure. It was a falling film type apparatus provided with a rotating wiper that continuously rubbed the falling film on the evaporating surface. The undesirable free fatty acids and other volatile compounds were removed through this method. Free fatty acids and glycerides were identified by the Gas – Liquid Chromatographic method and standard column chromatographic IUPAC method respectively.

## III.RESULT AND DISCUSSIONS

Table 1 shows the analytical characteristics and fatty acid composition of SBO DD. It contains about 73.7% FFA, 13.3% unsaponifiable matters which contains tocopherols (4%), sterols (5.4%) and hydrocarbons (3.9%), 11.7% neutral glycerides. Regarding fatty acid composition, Table 1 shows that SBODD contains much higher amount of unsaturated fatty acid (79.4%) than saturated fatty acids (20.3%). Among unsaturated fatty acids, it contains 28.1% oleic acid, 49.2% linoleic acid and small amount of linolenic acid. Regarding saturated part, it contains 16.7% palmitic acid and 3.6% stearic acid.

TABLE I  
ANALYTICAL CHARACTERISTICS AND FATTY ACID COMPOSITION OF SBODD

Component	Amount (% w/w)	Component	Amount (% w/w)	Component	Amount (% w/w)
FFA (Total)	73.7±1.87	Neu. Glycerides	11.7±0.68	Unsap. Matters	13.3±0.88
Palmitic acid	16.7±0.62	MAG	2.3±0.68	Tocopherols	4.00±0.09
Stearic acid	3.6±0.11	DAG	3.3±1.87	Sterols	5.4±0.11
Oleic acid	28.1±0.97	TAG	6.1±1.87	Hydrocarbons	3.9±0.07
Linoleic acid	49.2±1.13				
Linolenic acid	2.1±0.08				

Values are represented as mean ± S.D. n=3

For the production of functional foods, the SBODD was treated with glycerol in different proportions in the presence of non-specific immobilized lipase NS 40013 (*Candida antarctica*). The different molar ratios of SBODD and glycerol maintained was 3:1, 3:1.1, 3:1.2 and 3:1.4 to study the esterification reaction for four different products A, B, C and D. Fig. 1 shows the progress of reaction for 8 hrs which indicates that as the glycerol concentration increases, more fatty acids are consumed. After 8hrs of reaction, the product A, B, C and D contain 14, 6.2, 3 and 1.4% FFA respectively.

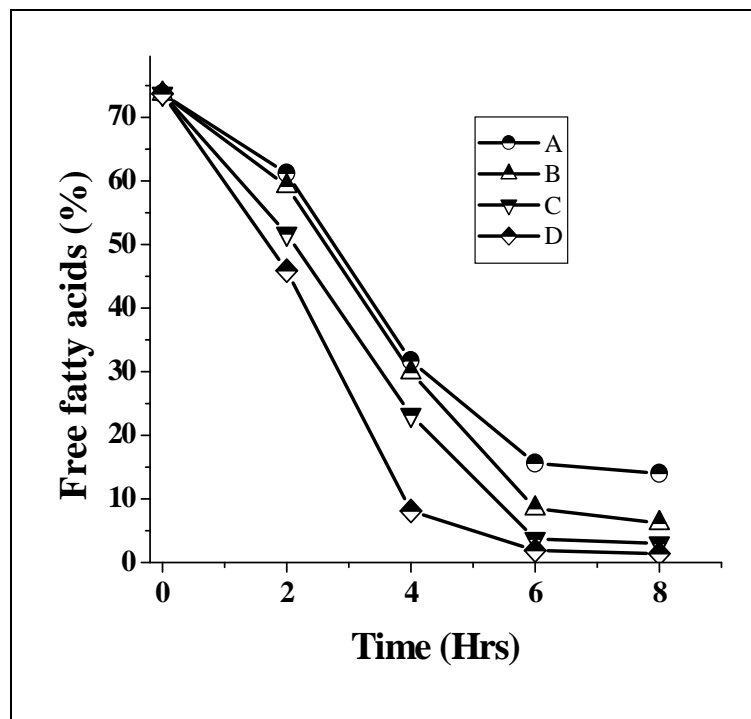


Fig. 1 Enzymatic glycerolysis of SBODD for functional foods.

[Enzyme used: lipase NS 40013 (*Candida antarctica*), Temperature:  $63 \pm 2^\circ\text{C}$ , Time: 8hrs]

The compositions of the purified products after molecular distillation are depicted in Table 2. Product A, B, C and D contained 42.1, 42.0, 41.7 and 32.5% TAGs, 25.4, 29.9, 36.0 and 45% DAGs and 12.2, 11.9, 9.9 and 9.4% MAGs respectively. Also, product A, B, C and D contained 12.5, 11.2, 10.1 and 9.6% unsaponifiable matters respectively.

TABLE 2  
COMPOSITION OF BIOESTERIFIED AND PURIFIED PRODUCTS

Product	TAG (% w/w)	DAG (% w/w)	MAG (% w/w)	Unsap. Matters (% w/w)
A	42.1 $\pm$ 0.97	25.4 $\pm$ 0.12	12.2 $\pm$ 0.32	12.5 $\pm$ 0.26
B	42.0 $\pm$ 0.89	29.9 $\pm$ 0.35	11.9 $\pm$ 0.24	11.2 $\pm$ 0.19
C	41.7 $\pm$ 0.73	36.0 $\pm$ 0.61	9.9 $\pm$ 0.11	10.1 $\pm$ 0.24
D	32.5 $\pm$ 0.22	45.0 $\pm$ 0.75	9.4 $\pm$ 0.09	9.6 $\pm$ 0.31

Values are represented as mean  $\pm$  S.D. n=3

From Table 2, it can be concluded that product A, B and C can be considered as TAG rich functional foods and product D can be considered as DAG rich functional foods. So by simply varying the concentration of glycerol in the reaction mixture, products of desired composition can be manufactured. Moreover, TAG/DAG rich products along with higher amount of unsaponifiable matters are regarded as functional foods with required commercial value. Fig. 2 shows the distribution of tocopherols, sterols, hydrocarbons and others in the unsaponifiable matters present in the products A, B, C and D. From Fig. 2, it can be concluded that all the products contain highest amount of sterols in unsaponifiable matters. Product A and B contain higher amount of tocopherols than hydrocarbons but in product C and D, the amount of tocopherols are almost similar as the amount of hydrocarbons.



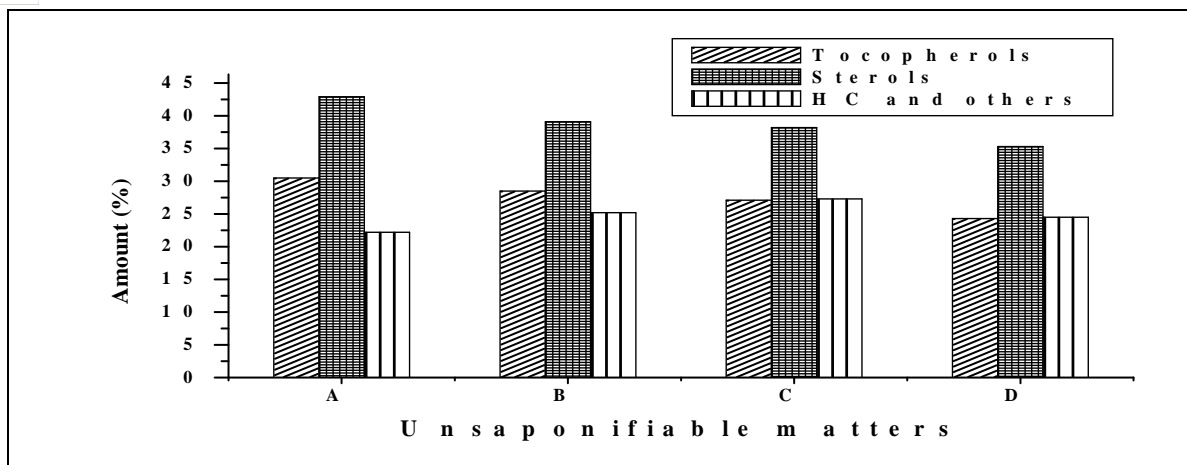


Fig.2 Distribution of unsaponifiable matters in the products A, B, C and D.

Table 3 shows the quality of functional foods on the basis of acid value, peroxide value, anisidine value and colour. By efficient molecular distillation at high vacuum, it is possible to reduce the FFA content and other volatile odoriferous compounds to a minimum level. The products A, B, C and D have acid value <0.1, peroxide value <1, anisidine value 0.6-1.1 with a colour range of acceptable value.

TABLE 3  
QUALITY PARAMETERS OF THE FINAL PRODUCTS

Product	Acid value	Peroxide value	Anisidine value	Colour (Lovibond 1inch cell)
A	<0.1	<1	0.6	0.5Y+0.7R
B	<0.1	<1	0.7	0.4Y+0.6R
C	<0.1	<1	1.1	0.6Y+0.5R
D	<0.1	<1	1.1	0.8Y+0.7R

#### IV. CONCLUSIONS

Functional foods with significant amount of neutral glycerides and considerable amount of sterols, tocopherols and hydrocarbons can be produced from cheap raw materials like soybean oil deodoriser distillate in the presence of non specific enzyme NS 40013. So microbial lipase technology can be utilised for the production of functional foods from a relatively inferior grade raw materials and this technology also can be useful for the utilisation of vegetable oil refinery by products in a better and suitable way.

#### V. ACKNOWLEDGEMENT

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