J Am Oil Chem Soc (2010) 87:321–326
DOI 10.1007/s11746-009-1499-8

ORIGINAL PAPER

# Physicochemical Characteristics and Composition of Indian Soybean Oil Deodorizer Distillate and the Recovery of Phytosterols

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Received: 5 June 2009 / Revised: 15 October 2009 / Accepted: 21 October 2009 / Published online: 14 November 2009


**Abstract** The deodoriser distillate (DOD) of Indian soybean oil obtained from two industries processing soybean oil was investigated for its physicochemical characteristics, its composition of tocopherols, phytosterols, fatty acids and recovery of phytosterols for use in nutraceutical products. It was found that the two DOD samples studied were dark in color and had higher amounts of free fatty acids (22.7 and 49.9%), unsaponifiable matter (11.8 and 21.9%) (5–10 times found in soybean oil), total tocopherols (1957–2256 mg/100 g) (20 times the amount in soybean oil), and 6–10% of phytosterols (12–20 times the soybean oil). The fatty acids found were palmitic (23.2–25.5%), stearic (1.4–2.4%), oleic (23.8–26.1%), linoleic (40.4–41.1%) and linolenic (2.7–3.2%) acids. The unsaponifiable matter (21.9%) and phytosterols (8.7%) content of DOD-2 were higher than in DOD-1 and hence was more suited for isolation of phytosterols. Using hexane and water for crystallisation, the DOD-2 yielded a phytosterol fraction with lower recovery of 13.2–17.8% while treatment with alkali to remove FFA and the glycerides followed by organic solvent extraction yielded unsaponifiable matter containing phytosterols with a recovery of 74.6%. Later the unsaponifiable matter was purified by double crystallisation into a mixture of phytosterols of 87% purity containing $\beta$-sitosterol (34.3%), stigmasterol (3.1%) and campesterol (50.1%). The product may find use in foods, pharmaceuticals, cosmetics and allied industries probably as a nutraceutical.

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**Keywords** Crystallising · Deodoriser distillate · Physicochemical characteristics · Phytosterols · Soybean oil

## Introduction

Plant sterols and stanols (referred to collectively as phytosterols) are normal constituents of the human diet. Plant sterols such as campesterol, $\beta$-sitosterol and stigmasterol occur widely in plants in variable amounts [1]. The average daily intake of phytosterols in western countries is approximately 250 mg/day mainly derived from vegetable oils, cereals, nuts, etc. [1]. Plant sterols being minor bioactive lipid constituents (vegetable oil) have a positive effect on human health. Vegetable oils per se contain only 0.1–1% of phytosterols. However, a dose of 1–3 g of phytosterols per day could provide health benefits [2–5] which require phytosterols in a concentrated form and of food grade. Therefore, there is increased interest in the preparation of food enriched with phytosterols for use as a nutraceutical to provide such a health benefit [1]. In human consumption of plant derived sterols, particularly $\beta$-, reduces blood pressure [2], serum cholesterol levels and the risk of chronic heart disease [3, 4]. Phytosterols also serve as intermediates for synthesis of hormonal sterols, $\beta$-sitosterol exhibits significant anti-inflammatory effect and antitumor properties [5] antioxidant and anti-polymerisation effects in vegetable oils [6].

While, phytosterols constitute a major portion of the unsaponifiable matter in most of the vegetable oils and in the deodoriser distillate (DOD), the obvious choice of the starting material for preparation of phytosterols is the DOD of the vegetable oil industry. DOD is a by-product fraction collected from the deodorisation, which is the last major step in the vegetable oil refining process. It is a complex

mixture of free fatty acids, mono-, di-, and triglycerides, sterols and their esters, tocopherols, hydrocarbons, pesticides, and break down products of fatty acids such as aldehydes, ketones, and oxidised triglycerides [7].

The technical literature contains information on the recovery of phytosterols [7–11], including chemical treatment, solvent extraction, crystallisation and molecular distillation procedures. Crystallisation has frequently been used to purify phytosterols from DOD. Brown [8] reported a phytosterols product prepared by a continuous two-stage liquid–liquid extraction (LLE) with solvent pairs of methanol and hexane, followed by crystallisation using acetone as the solvent at 4 °C for 24 h. By this approach, a phytosterols concentrate of 73% purity was obtained from soy DOD containing 6.5% phytosterols [8]. Saponification is also a common practice to concentrate phytosterols. The phytosterols are then isolated from the resulting concentrate mixture by crystallisation [8–11]. Crystallisation is a simple process and the low temperature used prevents heat sensitive components from being destroyed, but the processes are covered by patents. Therefore, there is a need to develop a simple, efficient and economical process to prepare phytosterols from DOD. With this background, the present study was carried out with the objective of first characterising the DOD of soybean oil followed by the development of a method for the preparation of phytosterols from the DOD by crystallisation for nutraceutical purposes.

## Materials and Methods

### Materials

The DOD of soybean oil, DOD-1 and DOD-2, were procured from two refineries processing soybean oil by chemical refining method [Shakthi Soya Ltd, Pollachi, Tamilnadu (India) and K.S. Oils Ltd, Morena, Madhya Pradesh (India)] which extract soybean oil from Indian grown soybean seeds and recover the meal , hence designated as Indian Soybean oil DOD. Standard fatty acid methyl esters, phytosterols (cholesterol, $\beta$-sitosterol, campesterol, stigmasterol) and tocopherols ($\alpha$-, $\gamma$-, and $\delta$-tocopherols) were purchased from the Sigma Chemical Co. (St Louis, USA). Solvents and chemicals used were of analytical grade.

### Physicochemical Characteristics

The colour of the samples was determined using a Lovibond tintometer in a 1-in. cell on the Lovibond scale in transmittance mode and expressed as (5R + Y) units. Free fatty acids value, peroxide value, saponification value, unsaponifiable matter and tocopherols + tocotrienols content were determined by AOCS methods [12].

### Determination of Fatty Acid Composition

The DOD (200 mg) was saponified using 2 mL of 0.5 N methanolic sodium hydroxide and after extracting the unsaponifiable matter, the aqueous layer was collected, acidified by adding 0.2 mL of HCl to break the soap to fatty acids. The fatty acids were recovered using 2 mL of hexane and the hexane extract washed with water until it was free of hydrochloric acid. The extract was evaporated to get fatty acids. The isolated fatty acids were esterified using 1 mL of boron trifluoride in methanol and analysed as their fatty acid methyl esters and the concentration expressed as relative % peak areas [13].

### Instrumentation

Fatty acid methyl esters prepared were analysed using a gas chromatograph (model GC-9A, Shimadzu corporation, Kyoto, Japan) equipped with a data processor (model CR-4A, Shimadzu Corporation, Kyoto, Japan) and provided with a FID detector. The column used was 3 m long × 3.3 mm id, coated with 15% diethylene glycol succinate on a Chromosorb WAW, 60–80 mesh. The equipment was operated under the following conditions: nitrogen 40 mL/min, hydrogen 40 mL/min, injector temperature 200 °C, detector temperature 220 °C and column oven temperature 180 °C. Identification of fatty acids was carried out using standard fatty acid methyl esters.

### Determination of Phytosterols

The phytosterol composition was determined according to Holen [14]. The HPLC system (model LC-10AVP, Shimadzu corporation, Kyoto, Japan) was fitted with a C18 (ODS) column 150 mm × 4.6 mm id and UV detector and the mobile phase consisted of methanol/water (99:1 by vol). The phytosterols were identified using standard sterols and quantitated using standard cholesterol.

### Preparation of Phytosterols from DOD

Two methods were followed for the preparation of phytosterols from DOD and only DOD-2 was used in both methods.

## Method 1

### Preparation of Crude Phytosterols Mixture and Pure Phytosterols by Crystallisation

Direct crystallisation of phytosterols from DOD-2 in hexane was used in the first method as it is simple to carry out.

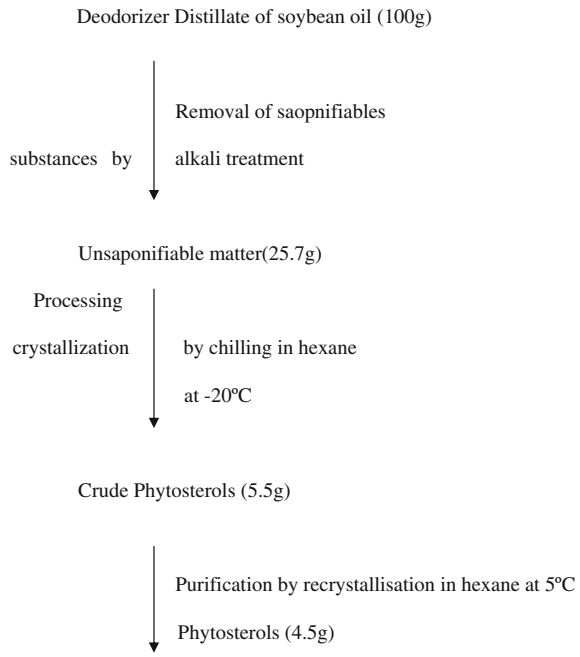


**Fig. 1** Flow sheet for isolation of phytosterols from DOD through saponification

The sample in triplicate (20-g amounts) was dissolved in hexane at different ratios (w/v) of 1:1, 1:3, 1:6 and 1:10. With 10% water (based on the weight of DOD) and cooled for 24, 48, and 72 h at −20 °C (Fig. 1). Afterwards, the contents were filtered and the residue on the filter paper was desolventised and air dried to get the crude phytosterols mixture. The yields and sterols content are provided for the 72-h cooling period with the maximum yield and recovery (Table 2). Then the crude phytosterols (9.2 g) from the 72-h batch with a 1:10 ratio was treated further with one more step of crystallisation with a 1:10 ratio of solvent (92 mL) and 10% water (0.92 mL) similarly at −20 °C for 24 h which was then filtered to get a white crystalline residue, named as a pure phytosterols mixture.

## Method 2

### Preparation of Phytosterols from Unsaponifiable Matter

A quantity of 20-g DOD-2 was placed in a 250-mL capacity flask, 20 mL of potassium hydroxide added, 120 ml ethyl alcohol was added (1:6 ratio) and warmed on a water bath, afterwards boiled with an air condenser on a boiling water bath for 2 h and then 80 mL of warm distilled water added followed by extraction with hexane (200 mL × 4). The hexane extract layer was collected and washed with water to free it from alkali, the hexane recovered in a rotavapor and traces of solvent removed under reduced pressure and the unsaponifiable matter of DOD (5.4 g) was then obtained.

### Preparation of Crude Phytosterols Mixture and Pure Phytosterols by Crystallisation

The unsaponifiable matter of the DOD (2.0 g × 2) was treated with 20 mL × 2 of hexane and 0.2 mL × 2 of water and mixed well using a stirrer for 30 min and then cooled at −20 °C for 72 h and then filtered to get a residue which is the crude phytosterols mixture (1.1 g) which after further crystallisation from hexane (11 mL) and water (0.1 mL) in the same ratio as that for DOD and chilled for 2 h at 5 °C. The crystallised product was separated by filtration and traces of solvent removed by air drying to get 1 and 0.95 g of crystalline phytosterols mixture.

### Statistical Analysis

All experiments were carried out in duplicate or triplicate and analysis carried out in duplicate and results were analysed for standard deviations using INSTAT statistical programme. The statistical significance was not calculated as there was no necessity.

## Results and Discussion

### Physicochemical Characteristics of DOD

The DOD-1 and DOD-2 of soybean oil were obtained from two companies and investigated for their characteristics before it being used for the extraction of phytosterols. Both the DOD were dark semisolid at room temperature and became liquid when heated to 50 °C. DOD-1 had a darker color (139.4 Lovibond color units), higher peroxide value (120.1 mequiv $O_2$/kg) and lower FFA content (22.7%) compared to DOD-2 which had colour, 75.4 Lovibond units, peroxide value 7.6 mequiv $O_2$/kg and FFA 49.9% (Table 1). The fatty acid compositions of DOD and FFA freed DOD were almost the same and were very different from the fatty acid composition of soybean oil (Table 3). The fatty acids found were palmitic (23.2–25.5%), stearic (1.4–2.4%), oleic (23.8–26.1%), linoleic (40.4–44.0%) and linolenic (2.7–3.2%) acids. The unsaponifiable matter, total tocopherols and phytosterols contents were 11.8%, 2246 mg%, and 7.8% for DOD 1 and 21.9%, 1957 mg%, and 8.7% for DOD-2, respectively. The range of values for the above parameters agrees with the literature reports [10, 15–20]. The higher saponification value for DOD-1 (166.5) and a lower FFA (22.7%) indicated the presence of a higher amount of glycerides (expressed as neutral oil, 65.5%) compared to DOD-2 (28.2%). The unsaponifiable matter of DOD-2 (21.9%) and phytosterols (8.7%) were higher than in DOD-1. Both the DOD had an off odour due to the presence of oxidised materials. The DOD-2 had a lower

**Table 1** Physicochemical characteristics of soy deodoriser distillate from two industries

| Parameters | DOD-1 | DOD-2 |
|---|---|---|
| Physical appearance (visual) | Dark semisolid | Dark semisolid |
| Color (1 in. cell, 5R+Y unit) | 139.4 ± 2.0 | 75.4 ± 1.0 |
| Free fatty acid value (as % oleic) | 22.7 ± 0.3 | 49.9 ± 2.4 |
| Peroxide value (mequiv $O_2$/kg) | 120.1 ± 3.2 | 7.6 ± 0.2 |
| Saponification value (mg KOH/g) | 166.5 ± 1.5 | 133.3 ± 3.9 |
| Neutral oil (%) | 65.5 ± 1.0 | 28.2 ± 1.1 |
| Unsaponifiable matter (%) | 11.8 ± 1.7 | 21.9 ± 1.2 |
| Total tocopherols (mg%) | 2256.0 ± 20.0 | 1957.0 ± 20.0 |
| Total phytosterols (%) | 7.8 ± 1.6 | 6.1 ± 0.3 |
| Fatty acid composition (relative area %) | | |
| Palmitic C16:0 | 23.2 ± 0.5 | 25.5 ± 0.5 |
| Stearic C18:0 | 1.4 ± 0.6 | 2.4 ± 0.7 |
| Oleic C18:$1_{(n-9)}$ | 26.1 ± 0.8 | 26.1 ± 0.6 |
| Linoleic C18:$2_{(n-6)}$ | 44.4 ± 1.8 | 40.4 ± 1.5 |
| Linolenic C18:$3_{(n-3)}$ | 3.2 ± 1.0 | 2.7 ± 1.1 |

DOD-1 from Shakthi Soya Ltd, Coimbatore (Tamilnadu), DOD-2 from K.S. Oils Ltd, Morena (Madhyapradesh)

Neutral oil % = [100 − (%unsaponifiable matter + %FFA)]

**Table 2** Effect of solvent ratio and cooling period on crystallisation of sterols from DOD-2

| Sample no.[a] | DOD:solvent ratio (v/v) | Cooling period (h) | Yield (%) | Phytosterols (%) | Recovery (%) |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 100 | 6.13 | 100 |
| 2 | 1:1 | 72 | 8.81 | 11.53 | 16.6 |
| 3 | 1:3 | 72 | 7.26 | 12.14 | 14.4 |
| 4 | 1:6 | 72 | 7.93 | 10.18 | 13.2 |
| 5 | 1:10 | 72 | 9.25 | 11.77 | 17.8 |

[a] All data are the means of three experiments

peroxide value (7.6 mequiv $O_2$/kg), lower neutral oil content (28.2%) and approximately similar phytosterols content (6.1%). Hence it was used for isolation of phytosterols.

## Preparation of Phytosterols from DOD-2 by Direct Crystallisation of DOD in Solvent (Method 1)

The crystallisation of phytosterols was carried out by dissolving the DOD-2 in hexane at different ratios (w/v) of 1:1, 1:3, 1:6 and 1:10 with water 10% w/w of DOD and cooled for 24, 48, and 72 h at −20 °C (Table 2). At ratios of 1:1–1:10, the phytosterols content did not change much and was in the range of 10.18–12.14% with a yield of 7.26–9.25% and a recovery of 13.2–17.8% for an incubation period of 72 h. The liquid fraction contained 78–80% of the remaining total sterols. The crude phytosterols solid fraction obtained in this fashion contained 60% free fatty acids and the yield of crystallised product was low at 10–12%. Whether the FFA was inhibiting crystallisation needs to be understood. Hence, another experiment was conducted where in, the FFA and neutral oil were removed by alkali treatment before crystallisation.

**Table 3** Phytosterols content and composition of the crude and crystallised products

| Parameter | Value Method 2 |
|---|---|
| Starting sterols content of DOD (%) | 6.1 ± 0.3 |
| Yield of crude product (%) | 5.5 ± 0.5 |
| Sterols content (%) | 46.7 ± 2.0 |
| Recovery (%) | 65.8 |
| Composition of crystallised product | |
| Yield (%) | 4.5 ± 0.1 |
| Sterols content (%) | 87.0 ± 2.0 |
| Stigmasterol (%) | 3.1 ± 0.4 |
| Campesterol (%) | 50.1 ± 0.5 |
| β-Sitosterol (%) | 34.3 ± 1.5 |

## Preparation of Phytosterols from DOD-2 by Crystallisation of Unsaponifiable Matter of DOD in Solvent (Method 2)

A scheme shown in Fig. 1 was evolved by low temperature crystallisation of the unsaponifiable matter in hexane to get the crude phytosterols mixture. This was further purified by crystallisation at 5 °C for 2 h using hexane and water (1:10:0.1 w/v/w) as the solvent system.

The unsaponifiable matter prepared from DOD using either alkali in isopropyl alcohol or alkali in ethyl alcohol by AOCS procedure showed destruction of tocopherols as indicated by 7030 and 2300 mg/kg as against the starting value for DOD of 19570 mg/kg. It has been reported in the literature that during saponification excess alkali is used which destroys tocopherols [7]. Hence, deacidification of the DOD to remove free fatty acids before extraction of tocopherols may not be possible. However, phytosterols are easily recrystallised after removal of the FFA and the triglycerides.

## Phytosterols Composition

The composition of the purified product was stigmasterol 3.1%; campesterol 50.1% and β-sitosterol, 34.3% (Table 3). The HPLC pattern of the sterols in starting DOD, its unsaponifiable matter, crude product and crystallised product are shown in Fig. 2. The crystallised product did not have bad odour and can be used in foods, pharmaceuticals and cosmetics.

Recovery of sterols from DOD has been carried out in most processes through the removal of fatty acids. Crystallisation has frequently been used to purify phytosterols from DOD. Brown [8] reported a 73% sterols concentrate preparation by continuous two-stage LLE with solvent pairs of methanol and hexane, and then followed by crystallisation using acetone as a solvent at 4 °C for 24 h. In the present study, a phytosterols product with 87% purity could be prepared starting from a DOD containing 6.1% phytosterols by the unsaponifiable matter crystallisation approach.

Hirota et al. [10] have purified steryl esters and sterols from DOD of soybean oil by molecular distillation. The feed contained 10.4% tocopherols, 10.3% sterols and 12.8% steryl esters [10]. Jiang and Wang [15] determined the phytosterols composition in cereal by-products, with $\beta$-sitosterol amounting to 1.4–18.9% in oat bran, rice bran, wheat bran and corn fiber oils. Thanh et al. [16] reported that in sunflower oil (1232–1474 mg/kg) and olive oil (1472–1678 mg/kg) $\beta$-sitosterol was found as the major phytosterol. Soybean oil also contained 3270 mg/kg of $\beta$-sitosterol as the major phytosterol [17]. Verleyen et al. [18] have reported the free sterols and esterified sterols content of coconut, corn, cottonseed, olive, palm, peanut, rapeseed, soybean, sunflower, and walnut oils. They reported $\beta$-sitosterol 525 mg/kg as steryl esters and 1379 mg/kg as free sterols in crude soybean oil and $\beta$-sitosterol was found to be the major phytosterol in soybean and other vegetable oils studied by them. In the present study, the composition of the starting DOD, its unsaponifiable matter, crude phytosterols and purified product was stigmasterol: 0.84, 0.79, 2.48 and 3.1%; campesterol: 0.79, 3.20, 33.68 and 50.1%; and $\beta$-sitosterol: 1.77, 8.06, 22.75 and 34.3%, respectively. The DOD sample dissolved in hexane when separated on a silica gel column (10 g silica gel of 5% moisture of 60–120 mesh size) into a hexane eluted fraction and a 10% alcohol in hexane eluted fraction, the latter fraction contained 2.5% of stigmasterol, 34% of campesterol and 23% of $\beta$-sitosterol. The reason for a higher concentration of campesterol rather than $\beta$-sitosterol is not known. Probably, the purification method has an effect on the composition of the final product.

Reviews on the antioxidant activity and heat stability of phytosterols and their applications in different food products such as spreads, yoghurt, fruit juices including fruit nectars and tomato juice, healthy functional foods, beverages and also cooking oils with added phytosterols have been reported [6, 21–23]. It has been reported that stable phytosterol-rich cooking oil could be used to produce cholesterol lowering chips, other snack foods, as well as cakes and breads [22]. In the world market, such products are being marketed to restaurants and food service companies that are looking for more ways to manage cholesterol without drugs [22, 23].

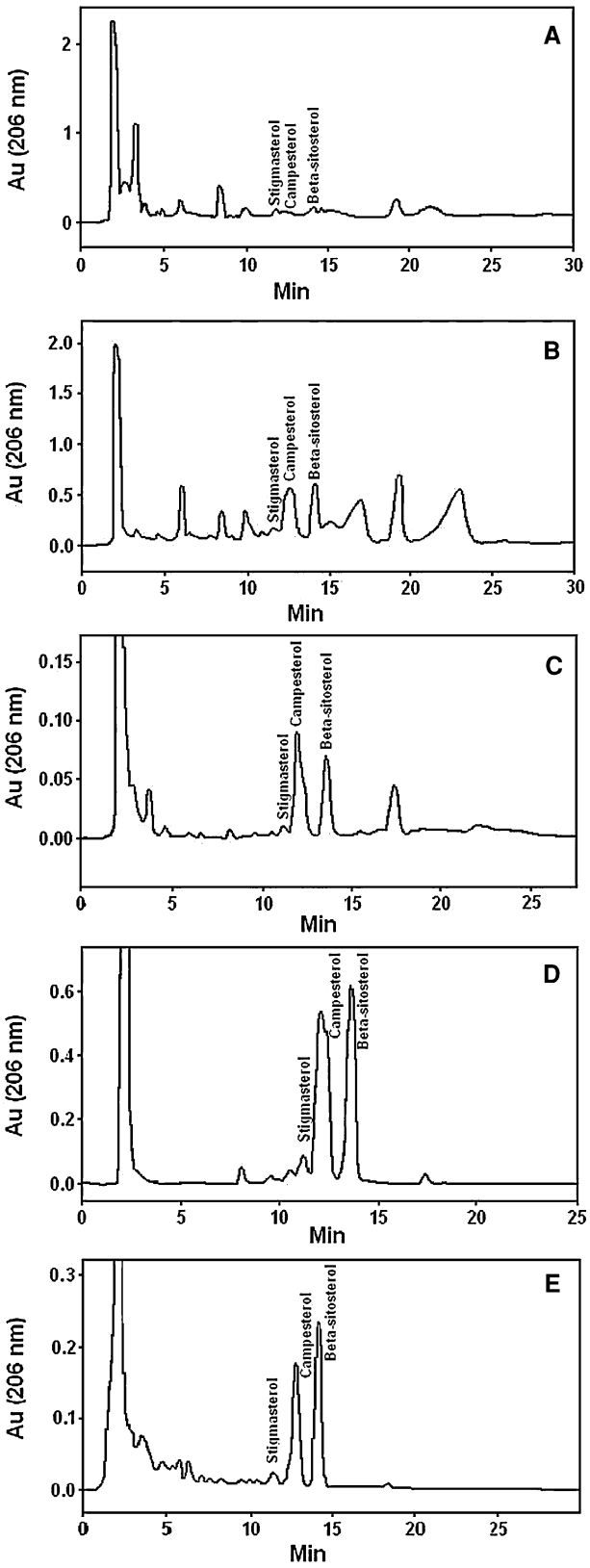


**Fig. 2** HPLC pattern of sterols of **a** starting DOD, **b** its unsaponifiable matter, **c** crude product, **d** crystallised product, and **e** standard mixture of phytosterols

**Acknowledgments** The authors are thankful to Dr. V. Prakash, Director of the Central Food Technological Research Institute, Mysore, for his keen interest and for providing infrastructural facilities and to Dr. B.R. Lokesh, Head, Lipid Science and Traditional Foods Department, for helpful discussions during the course of this work.

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