Production of phytosterols mix from palm fatty acid distillate (PFAD) through multi-staged extraction processes

J Nor Faizah1 *, A W Noorshamsiana1, W H Wan Hasamudin1, A A Astimar1, H Kamarudin1 and M T Ab Gapor2

1Engineering and Processing Research Division, Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia
2No. 40, Taman Mas Dua, Batu 9 Jalan Cheras, 42300, Selangor, Malaysia.

*Corresponding author: norfaizah@mpob.gov.my

Abstract. Phytosterols is one of bio-active compounds that naturally present in vegetable oils and its by-products or derivatives. The source of phytosterols can be in the form of by-product that generated during the extraction of vitamin E and in this study known as phytosterols resources (PSR). Vitamin E (tocopherols and tocotrienols) extracted from the palm fatty acid distillate (PFAD) produces solid by-product containing 2–4% (w/w) sterols. However, there are no suitable extraction and purification processes developed to exploit these compounds. Therefore, the extraction of phytosterols from PFAD by-product in a mini-pilot scale involving multistage extraction processes, which are solid-liquid extraction, saponification, liquid-liquid extraction and crystallization was developed. Phytosterols was recovered from the extraction and purification processes were of more than 80% purity and 80% yield, composed of β-sitosterol (21–22%), campesterol (13–20%) and stigmasterol (59–64%). In conclusion, this extraction process is technically feasible to extract and produce crude phytosterols from a by-product of the PFAD processing.

1. Introduction

Phytosterols (plant sterols) is one of bio-active compound that naturally synthesized in vegetable oils and its by-products or derivatives. Several types of phytosterols have been identified and reported but only β-sitosterol, campesterol and stigmasterol exist in significant amount in these resources [1, 2]. Other types of phytosterols such as brassicasterol, Δ5-avenasterol, Δ7-avenasterol, sitostanol, campestanol and Δ7-stigmasterol can also be found in minor quantities [3, 4].

Commercial plant sterols are extracted from soybean oil, corn oil, rapeseed oil, sunflower oil as well as tall oil. In palm oil, phytosterols can be found as a minor component together with tocotrienols, tocopherols, carotene, co-enzyme Q10 and squalene [5]. The phytosterols content in crude palm oil (CPO) is ranging from 250 to 730 ppm [6]. Phytosterols also present in the by-product of palm oil mill and refinery (Figure 1) such as palm pressed fibre oil (PPFO) and palm fatty acid distillate (PFAD) with various concentrations depending on the processes [7–9]. In Malaysia, it was reported that about 770 000 tons of PFAD has been generated in 2017 [10]. In average, phytosterols content in PFAD was 0.4 wt% [7] and from this figure, it can be estimated about 3000 tons of phytosterols are available for extraction from the PFAD.
Figure 1. Flowchart of oil palm fresh fruit bunch (FFB) and crude palm oil (CPO) processes with main products and by-products

Phytosterols can be extracted using various methods and extraction technologies depending on the source of raw materials such as vegetable oils, tall oils or waste from both oil types [3]. The common recovery processes of minor components (e.g. vitamin E, carotene, phytosterols and squalene) from oil include esterification, saponification, molecular distillation, crystallization and filtration [6, 11]. Greener method which is supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) technology that were introduced to extract oil enriched with phyttonutrient from PPFO, olive oil deodorizer and PFAD [8, 12-14]. The green SFC and SFE system use carbon dioxide (CO$_2$) as a solvent which is nontoxic, inexpensive, nonflammable, and nonpolluting solvent for the extraction of the minor components. Both systems that described before are able to recover all minor components from the feedstock, however, it produces low purity of the individual type of minor components. For instance, phytosterols concentration recovered was low at below 20%. Therefore, the extraction system requires additional processes in order to obtain high purity of individual phyttonutrient such as solvent fractionation [14]. Moreover, compared to conventional processes, these new technologies incur very high capital cost and need high skills manpower.

Meanwhile, the commercial Vitamin E production from PFAD also produced solid by-product or residue that still contained unrecovered phytosterols, but the extraction and concentration process has yet to be developed. Thus, this paper is aimed to study the technical feasibility of multi-staged extraction for the production of PFAD-based phytosterols mix in mini pilot scale. The recovery of this valuable minor component from the by-product of oil palm processing will add value to the oil palm industry and thus make the industry more sustainable.

2. Materials and Method

2.1. Materials
PFAD was purchased from a refinery company; MOI Foods Malaysia Sdn Bhd, Selangor, Malaysia. The samples are stored in stainless steel drum before the extraction processes. Chemical such as 100% methanol, 100% n-hexane, 100% acetone, 98% sulphuric acid, 100% sodium hydroxide pellet, 95% denatured ethanol, 100% n-heptane and ion exchange resin were purchased from local chemical companies.

PFAD is then subjected to numerous processes as described by Ab Gapor et al. (1993) for the vitamin E extraction process [15]. The process includes the crystallization of concentrated PFAD ester that has produced solid by-product. The solid residue (or by-product) from this process is collected and analyzed for phytonutrient content. This by-product called as phytosterols resources (PSR).

2.2. Methods

2.2.1. Extraction of phytosterols from PSR. The extraction and concentration process of phytosterols from the PSR was conducted through multi-staged processes that are solid-liquid extraction (SLE), saponification, liquid-liquid extraction (LLE) and crystallization. The operating parameters used as illustrated in Figure 2. All unit operation used for extraction process was in mini-plant scale with the capacity between 5 L and 10 L. Four types of solvent have been tested in laboratory scale crystallization, which are ethanol, methanol, acetone and hexane.

![Figure 2. Process flow and operating parameters of phytosterols extraction from the phytosterols resources (PSR)]
2.2.2. Analysis. All samples were pre-treated via saponification reaction prior to the analysis of the phytosterols content based on MPOB test method [16]. The treated samples were analysed using gas chromatograph system (Autosystem XL, Perkin Elmer) with flame ionization detector (FID) and SAC™-5 (Sigma) column for quantification of phytosterols concentration. The initial oven temperature, maximum oven temperature and detector temperature used is 270 °C, 320 °C and 270 °C respectively.

For squalene analysis, samples were dissolved and diluted in 100% n-hexane to an appropriate concentration. The analysis was carried out using ACQUITY™ UPLC (Waters) with ACQUITY™ UPLC BEH (Waters) reverse phase C18 column and photodiode array (PDA) detector. The detection wavelength is 220 nm with total analysis time of 10 minutes. The detection of vitamin E (tocopherol and tocotrienols) was carried out in Hewlett Packard HPLC that equipped with normal phase Luna® 5U (Phenomenex) normal phase silica column and fluorescence detector with excitation of 295 nm and emission of 300 and total analysis time of 30 minutes.

Fourier Transform Infrared (FTIR) analysis was conducted using Perkin Elmer Spectrum One FTIR Spectrometer with wavelength in the range of 4000 – 650 cm⁻¹. The spectra were compared with the individual phytosterols standard. Finally, Nuclear Magnetic Resonance (NMR) analysis with ¹H and ¹³C experiments were performed using 600 MHz JEOL Spectrometer for compound identification and confirmation.

3. Results and Discussion

3.1. Analysis of phytonutrient content in PFAD and PSR

Initially, the total phytosterols content in PFAD was in average of 0.31 wt %. From Table 1, it shows that the PSR which is the solid residue of Vitamin E extraction plant composed of up to 15.15% of unsaponifiable matter (USM) and 14.10% of phytosterols. The PSR also contained squalene in the range of 2.49% to 3.38%. Chandrasekaram (2009) had produced phytonutrient concentrate as feedstock for minor component extraction from palm methyl ester. Nevertheless, USM in palm phytonutrient concentrate produce was about similar to the PSR (10% content), but the phytosterols content in PSR is relatively higher as compared to palm phytonutrient concentrate [17]. Most of USM in phytonutrient concentrate were contributed by vitamin E (8.99%) and carotene (20.99%). While in PSR, the vitamin E was not detectable by the HPLC analysis.

Table 1. Comparison of total unsaponifiable materials and its phytosterols content in PFAD, PSR and phytonutrient concentrate

| Samples                                    | PFAD  | PSR        | Phytonutrient concentrate³ |
|--------------------------------------------|-------|------------|----------------------------|
| Unsaponifiable matter, USM (%)            | 1.86 - 2.87 | 3.82 – 15.15 | 10                         |
| Phytosterols content in USM (%)            | 10.31 – 15.33 | 61.59 – 86.53 | 3.71                       |
| Phytosterols composition in USM (%)        |       |            |                            |
| Cholesterol                                | 3-9   | 1-2        | 6                          |
| Campesterol                                | 23-25 | 22-23      | 34                         |
| Stigmasterol                               | 13-14 | 15-16      | 22                         |
| β-Sitosterol                               | 53-60 | 60-61      | 39                         |
| Total phytosterols in samples (ppm)        | 600 – 4200 | 20,300 -141,000 | 1.59                       |
| Squalene (ppm)                             | 3200 - 5600 | 24,900 – 33,800 | 8110                       |
| Total Vitamin E (ppm)                      | 3500 - 5000 | N.D        | 89,940                     |
| Carotene                                   | N.A   | N.A        | 209,880                    |

³Source : Chandrasekaram, 2009
bNormalized to 100%
After subjecting the PSR into SLE process, LLE process and saponification reaction, most of non-polar compounds including phytosterols, squalene and traces of vitamin E were concentrated and recovered in the phytosterols-rich fraction 1 and 2 (PSRF-1 and PSRF-2). The yield of PSRF-2 was in the range of 14.32% to 19.95%. The phytosterols content were between 36.50 wt % and 61.45 wt %. Apart from phytosterols, the PSRF-2 also contained squalene and traces of unreacted glycerides. The components of PSRF-2 are having different melting points. Hence, low temperature solvent crystallization was applied to isolate the phytosterols from the PSRF-2 mixture.

As mentioned before, four types of solvent have been tested in laboratory scale crystallization for the phytosterols purification. Crystallization of USM with hexane gave highest yield which is 62% with pure phytosterols (100% purity). Then it followed by ethanol and then acetone with yield of 43% and 32% respectively. The purity of phytosterols obtained by crystallization of USM with ethanol and acetone were 93.92% and 100% respectively. The lowest crystal yield obtained in methanol solvent that is 25% with phytosterols content of 48.25 wt %. Other solvent such as benzene, toluene and cyclohexane may gave higher phytosterols purity, but with lower yield and the process was highly toxic [18]. Then, the mini pilot scale crystallization process was conducted using two most effective solvents which are hexane and ethanol with similar crystallization parameters as described in method section. The yield and purity of extracted phytosterols in mini pilot scale using hexane solvent were 47% and 87.23% respectively. These values were lower than the laboratory scale extraction but still satisfy the purity of crude phytosterols which is more than 80%. Others impurities contributed by traces of squalene (less than 1%) and also unreacted glycerides that have closer melting point as phytosterols.

The mini pilot scale set up for the multistage extraction process is depicted in Figure 3.
The concentrations of total phytosterols and the yield for each extraction stage are tabulated in Table 2.

Table 2. Concentration of total phytosterols and yield for each extraction process

| Sample name | Sampling point / unit operation | Concentration of Total Sterol (wt %) | Yield (%) |
|-------------|---------------------------------|-------------------------------------|-----------|
| PFAD        | Raw material                    | Minimum: 0.2 Maximum: 0.42 Mean: 0.31±0.07 | -         |
| PSR         | Solid by-product from Vitamin E plant | Minimum: 2.03 Maximum: 8.54 Mean: 7.51±0.27 | 1.50      |
| PSRF-1      | Solid-liquid extraction (SLE) system | Minimum: 3.95 Maximum: 8.61 Mean: 8.03±0.35 | 91.50     |
| PSRF-2      | Saponification reactor and LLE extraction system | Minimum: 36.5 Maximum: 61.45 Mean: 47.64±4.78 | 16.70     |
| Final product | Crystallization and filtration system | Minimum: 84.59 Maximum: 94.05 Mean: 87.23±5.70 | 47.32     |

Based on FTIR analysis, the absorption includes 1641.6 cm⁻¹ for the olefinic bond in stigmasterol, however, the band was absorbed weakly due to the C=C stretching (Figure 4). The absorption frequency at 1051.89 cm⁻¹ and 958.79 cm⁻¹ are for trisubstituted olefin which the most characteristic bands for β-sitosterol and stigmasterol [19]. The ¹³C NMR spectrum (Figure 5) shows that the presence of the compound in phytosterols mixture is in a form of steroid skeleton due to the similar chemical shift obtained as compared in the literature [19, 20]. The ¹³C NMR spectrum for campesterol and β-sitosterols were identical, thus, the presence of those two compounds were confirmed.

![FTIR Spectrum for PFAD-based phytosterols, cholesterol standard and stigmasterol standard](image)

Figure 4. IR Spectrum for PFAD-based phytosterols, cholesterol standard and stigmasterol standard
4. Conclusions

Extraction and purification of phytosterols from solid residue obtained after vitamin E extraction from PFAD have been successfully conducted using multistage extraction processes in laboratory scale and mini-pilot scale. Technically, the multistage extraction and purification methods in mini-pilot scale which comprises of SLE process with hexane solvent at 35°C in 5 h, saponification reaction at ethanol reflux temperature of 80°C for 1 h, LLE process with hexane in 5 h, crystallization and filtration from 65°C to -5°C for 20 h, were capable to produce phytosterols mixture with purity up to 94 wt% with individual sterols compositions of β-sitosterol (21-22%), campesterol (13-20%) and stigmasterol (59–64%). The overall recovery for sterol from the phytosterols resources was 84%. Apart from the production of vitamin E and methyl ester (biodiesel) from PFAD, the recovery of this valuable minor component from the by-product of PFAD processing will add value to the oil palm industry and thus make the industry more sustainable and economical.

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