Fruit palm peel extract derived from supercritical CO_2 extraction as a potent source of bioactive compounds

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Abstract. Supercritical CO_2 extraction (S-CO_2) was used as a green technology to extract oil from the milled fruit palm peels as a sustainable feedstock. The process was carried out at five different temperatures (40, 50, 60, 70, and 80 °C), constant pressure of 25 MPa, a flow rate of 5 mL/min, and a co-solvent ratio of 5% volume. An optimum oil extract yield of 3.95% was obtained at the highest temperature (80 °C) after 150 mins of extraction. Fourier Transform Infrared (FTIR) and Scanning Electron Microscope (SEM) were explored to characterize the chemical composition of the extract and simultaneously evaluated the presence of bioactive compounds using High-Performance Liquid Chromatography (HPLC). Phytochemicals present in the extracts, namely, quercetin and beta-carotene, were quantified as 1.1929 and 2.3354 mg/g, respectively, while there was no amount of gallic acid detected in the extract.

Introduction

Oil palm fruit is a monocotyledonous plant belonging to the palm family Palmae, which comprises *E. guineensis,* and *E. oleifera*, with suitable and different morphological traits [1]. Palm oil is increasingly becoming a demanded commodity worldwide, particularly for use in food products and other applications, and as a revenue generator through export. Presently, Indonesia and Malaysia are the major palm oil producers, accounting for 56% and 31% of total global production, respectively [2]. Studies have shown that about 42 million tons of global oil production are contributed by palm oil, and this tabled it as the most traded vegetable oil that engulfed nearly 60% of the global oil exports [3,4]. The phenomenon compelled the growing rate of palm oil plantations in most palm-producing countries and is expected to multiply by 2030, considering its substantial growing demand.

Notably, the palm fruits contained carotenoids and chlorophylls as naturally occurring pigments, with the fruit containing plenty of these phytochemicals than the oil. Therefore, the utilization of fruit palm peel extract as a source of bioactive compounds such as antioxidants, antimicrobials, and as a raw material for many industrial applications in food, pharmaceuticals, body care, biofuel, and other green chemicals is a promising option. However, the potency of the recovered value-added bioproducts and their stabilities depends on the plantpart, extraction technique employed and other preparatory conditions [5, 6]. Consequently, S-CO_2 extraction is placed forward as a clean and green extraction technique, that is environmentally friendlier, non-polluting, and toxic-free. This ruled out the drawbacks constituted by the conventional methods

such as time consumption, use of hazardous solvents, high temperature, and intensive energy demand.

Moreover, the non-polarity of the extracting solvent (CO2), exempted the possibility of oxidization which often affects the stability and bioactivity strength of the extracts obtained through conventional extraction methods. Hence, these are some of the merits that placed S- CO2 extraction forward as the most plausible technique for the extraction of bioactive compounds [7].

Material and methods

Materials. Fresh palm fruit was obtained as a gift from a palm tree farm in the Bota area, Perak-Malaysia. The sample was washed to remove dirt, and other particle bounded, peeled off using stainless steel knife, and subjected to an oven (Memmert, UN55BO), drying at 50 ℃ for 72 hours[8]. Thereafter, pulverized milling machine (FRITSCH, V2A 1.4301) was used to grind the dried sample and sieved with a 0.5 mm size mesh. Next, the milled sample was transferred to an airtight bottle and stored in a refrigerator for upward extraction using S-CO2. Figure 1 shows the various preparatory steps of the mango peels. Besides, HPLC-grade chemicals such as beta-carotene (≥ 93%), quercetin (≥ 95%), gallic acid (97.5 %), and other high-purity solvents like methanol,acetonitrile, acetic acid, and trichloromethane were used in this research.

Fig. 1: Sample preparation steps.

S-CO2 Extraction. The milled fruit palm peels were extracted using S-CO2 extraction according to a procedure reported by Ruslan et al. [9], in a modified version. The extraction setup comprises the extracting solvent reservoir (CO2 supply tank) supplying liquid chilled CO2 (0 ℃) via conducting pipe to the extraction vessel inside the oven. The pumping rate typically controls the flow rate, while pressure drop is controlled by the automated back pressure regulator, ABPR (Jasco, BP-2080 Plus). Extraction conditions for the significant S-CO2 extraction parameters used were a pressure of 25 MPa, a flow rate of 5 mL/min, and a co-solvent ratio of 5% vol. Next, 5.0 g of the milled peel sample was loaded into the extraction vessel (Jasco, EV- 3-50-2), and the time was noted after the first released pressure purge from the ABPR was completed. Subsequently, the oil extract was collected in an amber glass bottle immersed in an ice bath for 30, 60, 90, 120, and 150 mins. Finally, the collected extracts were diluted with 2.0 mL of n-hexane and stored diluted extracts in a refrigerator at 4 ℃ for upward HPLC analysis. Consequently, the percentage extract yield (XX_0 %) of the milled fruit palm was calculated using equation 1.

$$X_0\ (\%) = \frac{Weight\ of\ extract\ obtained}{Weight\ of\ milled\ sample\ used}\ \text{X}\ 100 \quad (1)$$

HPLC Analysis. SHIMADZU 20 AD HPLC profiling, integrated with an ultraviolet/diode array detector (UV/DAD), was used to isolate three bioactive compounds, namely, beta-carotene, quercetin, and gallic acid in the fruit palm oil extract derived from S-CO2 extraction. In this work, a reversed-phase (RP) separation using a poroshell column, 120 EC-C18 (4.6 mm × 100 mm, 2.7 μm) was employed for this determination. Solvent compositions of acetonitrile/acetic acid and methanol/trichloromethane with a volume ratio of (98:2, v/v) each were prepared, filtered, degassed, and used as the mobile phases for the identification of the polyphenols (quercetin & gallic acid) carotenoids (beta-carotene) respectively, [10]. Before the analysis, the oil extracts were diluted with solvents using a 1:9 ratio, v/v to reduce their concentrations. Next, 3.0 mL of each of the diluted extracts and their respective bioactive compounds' external standard solutions were filtered using a 0.45 μm nylon syringe. Finally, 20 μL of each sample was used for injection into the HPLC, maintained at 40 ℃ column temperature, 1.0 mL/min flow rate, and 280 and 454 nm UV detector ranges for detecting quercetin, gallic acid, and beta-carotene, respectively.

Physicochemical characterization. The superficial structure of the milled banana peels was analyzed using SEM, Hitachi TM3030, Tabletop. The dried sample was stuck to the specimen stub with conductive tape [11]. Next, the specimen stub was tied to the specimen holder and placed into the specimen chamber. The micrographs were recorded at a medium magnification of 1000 x 100 μm. The sample extract was subjected to infrared spectral analysis within the range of 4000 – 650 cm^{-1} according to a procedure reported by Bello et al. [12], using Cary 630 Agilent Technologies.

Results and Discussion

Extract Yield. The experimental yield *(X_o)* results obtained for this work, at various temperatures of 40 – 80 ℃ over increasing extraction time from 30 to 150 min, are shown in Table 2. According to the results, the lowest extract yield was obtained at the initial time (30 min) while the highest was recorded at the maximum extraction time. Overall, the lowest and highest yields recovered were 2.61and 3.95%, obtained at 40 ℃, 30 min, and 80 ℃, 150 min, respectively. This shows that the extract yield increases proportionally as the temperature rises from 40 – 80 ℃, and that role played by the extraction time follows the same trend. Therefore, in kinetic terms, it can be referred to that the rate of extract recovery from the fruit palm peels using S-CO2 carried out in this work is a forward reaction [13]. However, at the initial extraction time (30min), the difference in yield between the two boundary temperatures (40 and 80 ℃) is 0.21%.

On the other hand, a difference of 0.74% yield was recorded between the same temperature gap at the final extraction time of 150 min. This justified that prolonging extraction time from 30 – 150 min, has a direct impact on the recovery of the extract, where an increment in the extract yield by 0.53% was recorded. Looking at the impact of temperature on the recovery of the oil extract over time shown in Figure 2, the yield increases linearly as the temperature rises over the respective extraction times. However, at the lowest temperature (40℃), the difference in yield between the initial extraction time (30 min) and the final extraction time (150 min) is 0.6%. While the difference in yield between the same extraction time boundary (30 - 150 min) at the highest temperature (80 ℃), is 1.13%. This significant difference in yield between the highest and lowest temperature over the same extraction time range can be explained based on the vital role played by temperature in reducing solvent density and enhancing solubilization of the solute by the extractant (CO2) solvent. Hence, this trend for the effects of temperature on the oil extract yield observed is in line with the conditioned theory of S-CO2 which stipulates that the yield of the extract generally increases at higher temperatures due improve solute mass transfer into the plant matrices [14]. Consequently, the obtained experimental extract yield ranging from 2.61 - 3.95% was appreciably higher, especially in the context of S-CO2 extraction. This must be connected to the greater molecular interactions between the compounds present in the fruit palm peel extracts (carotenoids) and the non-polar CO2 (extracting solvent), which utterly dissolves the compounds due to the similarity law of like dissolve like since the carotenoids were also non- polar [15].

Table 1: Results of fruit palm peel extracts derived from SCO_2 at different temperatures

		Extract yield over time (%)				
Samples	Time (min)	40 °C	50 °C	60 °C	70 °C	80 °C
Milled fruit palm peels	30	2.61	2.65	2.68	2.71	2.82
ü	60	2.72	2.78	2.85	3.00	3.18
ü	90	2.90	3.15	3.41	3.57	3.63
ü	120	3.15	3.41	3.68	3.76	3.82
ü	150	3.21	3.50	3.75	3.84	3.95

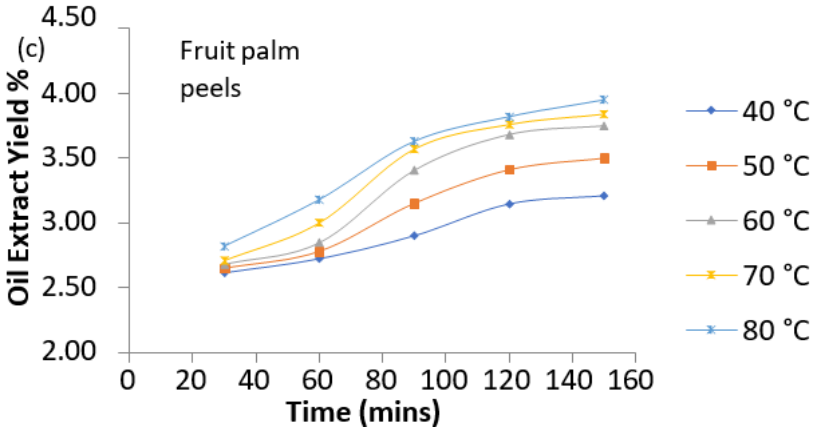

Fig. 2: Influence of extraction time and temperature on fruit palm peels oil extract yield

Quantification of bioactive compounds by HPLC. The disparity in the retention times, peak areas, chromatographic peaks of the spectra for the sample and those of the standard compounds, and the quantity of the targeted bioactive compounds was observed. However, the fitting of the regression curves shows good linearity with R2 values of 0.9754, 0.9955, and 0.9662 for quercetin, gallic acid, and beta-carotene, respectively (Table 2). The quantification of the targeted bioactive compounds, namely beta- carotene, quercetin, and gallic acid present in the oil extract, was carried out using the calibration curve determination method [16], where a graph of peak area (µV.s) on the Y-axis against concentrations (ppm) of the standard solutions on the X-axis was plotted. The generated calibration equations, peak areas, retention times, and the quantified amount of the respective target compounds are shown in Table 2. The estimated quantities of the target compounds in the oil palm extract samples calculated in milligrams per gram (mg/g) are 2.3354 and 1.1929 for the beta-carotene and quercetin, respectively. On the other hand, no amount of gallic acid was detected in this work. However, the result obtained in this work is in agreement with other previous research findings [17], establishing the presence of a higher number of carotenoids (5 – 7 mg/g) in fruit palm.

Table 2: Standard curve analysis of the external standards obtained by RP-HPLC–DAD, and quantified mass of the samples in mg/g

Standard compounds	Oil extracts	Retention time (min)	Peak area (µV.s)	Standard equation	R^2	Quantified extracts (mg/g)
Quercetin	-	2.563	16200537	$y = 16557x - 2x10^6$	0.98	-
	Fruit palm peels	2.581	194499	-	-	1.1929
Gallic acid	-	2.459	2772466	y = 45815x - 54305	0.998	-
	Fruit palm peels	ND	ND	-	-	ND
Beta-carotene	-	54.48	625881	y = 633.04x - 43379	0.97	
	Fruit palm peels	53.49	120890	-	-	2.3354

ND = not detected

Qualitative analysis using FT-IR. Absorption bands ranging between 2957.80 – 723.42 cm^{-1} were detected in the FT-IR spectrum of the fruit palm peel oil extract, as shown in Figure 3. A procedure reported by Barbara, [18] was followed in assigning the various vibrational modes. An absorption band at 1747.81 cm^{-1} could be assigned to C=C stretching consistent with Csp2 hybridized alkene that continued to overlap within the ring system. Another notable peak at 1462.51 cm^{-1} is linked to the C=C stretching in the phenyl groups attached to the two ends of the beta-carotene structure. Similarly, the appearance of low-intensity stretches at 1378.50 and 1242.83 cm^{-1} are for the methyl substituent symmetry (-CH3). Other absorption bands detected within the fingerprint region, precisely 1161.99 cm^{-1} and 1065.64 cm^{-1}, might be accorded to C-C skeletal having Csp3 hybridized. However, peaks spotted at 2957.80, 2923.42, and 2855.89 cm^{-1} are for OH stretches associated with traces of polyphenol (quercetin). Overall, the purity of the oil extract can be confirmed by looking at the excellent baseline resolution obtained in the spectrum [19].

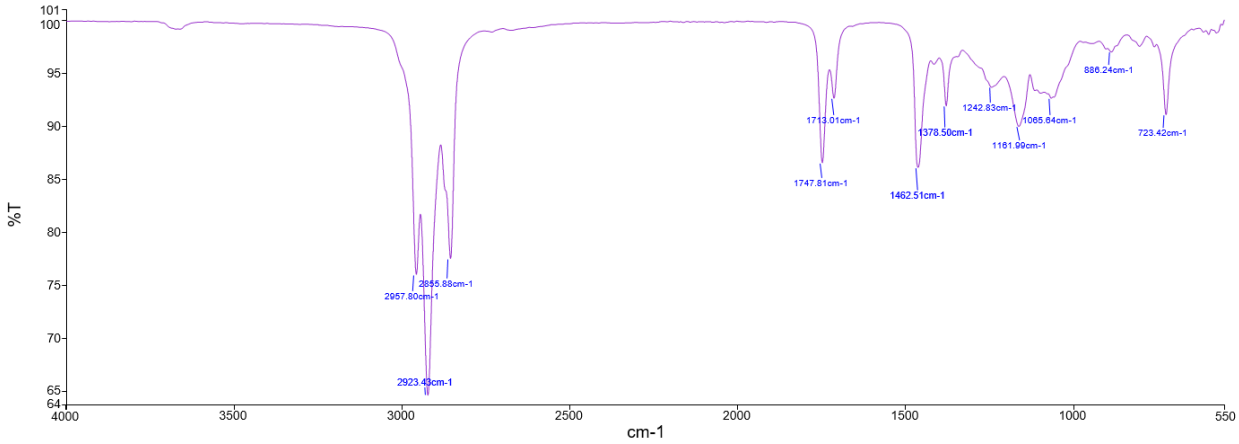

Fig. 3: FT-IR spectrum of fruit palm peel oil extract

Morphology Analysis of SEM. The surface morphology of the fruit palm peels before and after extraction studied using SEM, are presented in Figures 4 a and b, respectively. The images were viewed at a medium magnification of 1000 x 100 µm for both pre-and post-extracted samples. Comparatively, a smoother and relatively hydrated nature of the pre-extracted samples, appearing darker (Figure 1 and 4a) was due to unextracted oil surrounding the particle [20]. However, a more porous surface and lighter appearance (Figures 1 and 4b) of the post-extracted sample suggested the dissolution or rupture of the cell wall matrix and exit of oil that embedded the plant matrices [21]. To this end, a visible difference was observed concerning the physical appearance of the particle surface and colour. This feature was uniquely attributed to the selectivity of the SCO2 extraction process, where the extractable solutes broke down from inside the plant matrices due to high temperature and pressure compression leading to the withdrawal of oil extract. A similar observation was previously reported by Chai et al., [22] on the pre and post-extraction sample of papaya Linn. leaves using SCO_2.

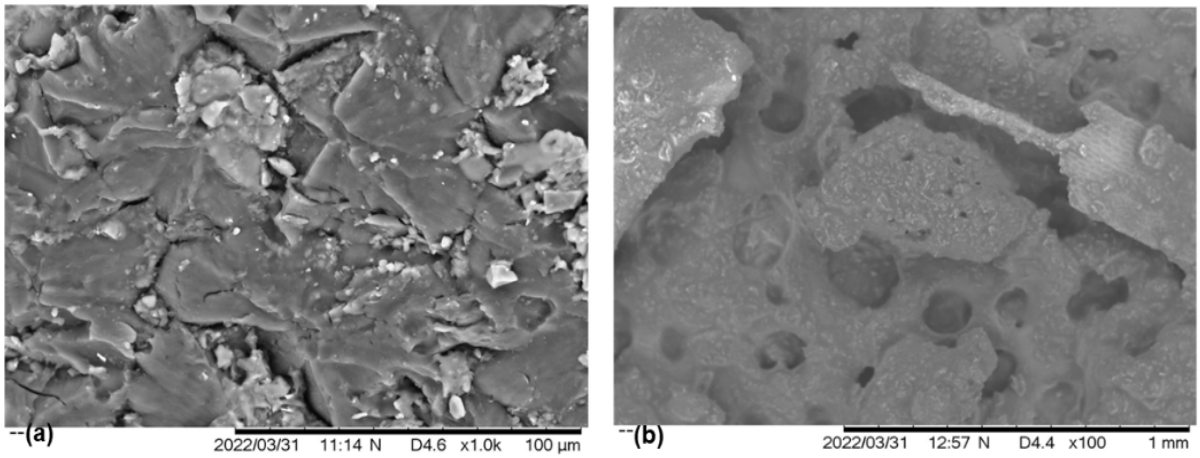

Fig. 4: Pre (a) and post-extracted (b) milled peel samples viewed at the same magnification

Conclusion

This work has explored using SCO2 as a green extraction technique to recover oil extract from fruit palm peels. Based on the results, the highest oil extract (3.95%) was recovered at the maximum temperature of 80 ℃ over a prolonged extraction time of 150 min. However, the lowest yield obtained was 2.61% at 40℃ after 30 mins of extraction. Thus, these results established that a forward reaction is favoured considering the symmetrical increase in the oil recovery rate as the temperature increases over time. Therefore, temperature significantly increases the oil yield rate by enhancing diffusivity and solubilization of the analyte from the inner pores of the plant matrices. Quantitatively, the amount of bioactive compounds analyzed using RP-HPLC-DAD shows that the extract contained a higher quantity of beta-carotene than quercetin and gallic acid. In the qualitative characterization, the purity of the obtained oil extract as claimed by the SCO2 technique was confirmed by the FT-IR analysis considering the good baseline resolution and absence of unwanted peaks. Moreover, the visible difference in the physical appearance of the pre-and post-extracted samples further attested to the uniqueness of the SCO2 technique.

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