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Research Article

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Identification and Quantification of Bioactive Compounds from Supercritical CO_2-Derived Extracts of Biomass Peel Wastes for Improving Biodiesel Oxidative Stability

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Abstract Biodiesel, a promising alternative biofuel derived from plant oils, suffers from a major drawback– poor oxidative stability. This means it readily reacts with oxygen in the air, which can lead to several problems, such as increased viscosity (affecting its cool flow), formation of damaging sediments (resulting from oxidation reactions forming sludge and gums that clog fuel filters and injectors, damaging engines), and degradation of fuel properties (such as fuel's lubricity, cetane number– a measure of ignition quality). For these reasons, this study explores the potential of natural antioxidants derived from biomass peel wastes (banana and mango peels) for improving biodiesel stability. Supercritical fluid extraction (SFE) using CO2 was employed to isolate bioactive compounds from these readily available biomass resources. The extraction parameters, including temperature (40-80°C) and time (30-150 min), were optimized to maximize extract yield, with mango peels yielded the highest amount (1.95%). High-performance liquid chromatography (HPLC) identified and quantified key antioxidant compounds like quercetin, gallic acid, and beta-carotene within the extracts. Hence, this research highlights the potential of repurposing biomass wastes as a sustainable and cost-effective source of natural antioxidants to enhance biodiesel oxidative stability.

Keywords Biodiesel, pollution, antioxidants, supercritical fluid, waste peel fruit

1. Introduction

The influx of environmental pollutions witnessed today is largely contributed by the growing rate of food waste generation particularly biomass waste from fruit and vegetable markets and juice processing factories. Indiscriminate discharge and improper management of these peel wastes led to the liberation of greenhouse (GHG) emissions like methane, carbon dioxide and nitrous oxide etc [1]. Surprisingly, little research progress has been made in redirecting the disposal of biowastes, particularly fruit peels to a reusable bioresources. Therefore, the idea of bringing back fruit peel wastes into the production circle as valuable bioresources is a strategy that aligns with the goal of supporting a greener and sustainable practices. Moreover, recovering phytochemicals compounds from these biowastes is a pathway to a sustainable and economically viable process

that would result in value-added products generation through the circular economy model [2]. Most importantly, the use of fruit peel wastes as potent sources of bioactive compounds like antioxidants is gaining researchers' attention because the phenolic compounds in plants are more soluble due to their increased polarity and sizeable chemical structures compared to their synthetic counterparts [3]. Other attracting features include zero-cost and having a higher proportion of phytochemicals than pulp and seed [4]. Inevitably, recovering the peel extracts using conventional extraction techniques has always been challenging due to their tied issues such as contamination by toxic solvents, phytochemical degradation due to high-temperature processing, and non-reproducibility leading to variations in the composition and quality of the extracts obtained from different batches of the same sample type [5]. Alternatively, SFE was explored as a green processing technique in this work to recover extracts from the milled fruit peels due to the techniques' superiority in terms of product purity, less energy-intensive, and toxic-free byproduct [2]. Altogether, these help in preserving heat-sensitive bioactive compounds against thermal degradation and solvent contamination.

2. Experimental Sections

2.1 Sample Identification and Preparation

The fruit varieties used in this work were identified as cavendish, and waterlily for the banana, and mango respectively. After identification, the fruit samples were washed, had their cap and other debris removed, and then cut to a smaller size using a stainless-steel knife. The sliced mesocarps were dried in an oven (Memmert UN55 BO) for 72 h at 50℃ with a tolerance of ±1 until the moisture content was reduced to 3-5%. The low-temperature drying method was chosen to prevent the degradation of the active phytochemicals in the sample [6]. After drying, the samples were ground into a powder using a power cutting mill (FRITSCH, V2A 1.4301) and sieved with a mesh size of 0.5 mm as shown in Figure 1. The ground sample of the banana and mango peels were stored in an airtight bottle in the refrigerator for extraction using SFE.

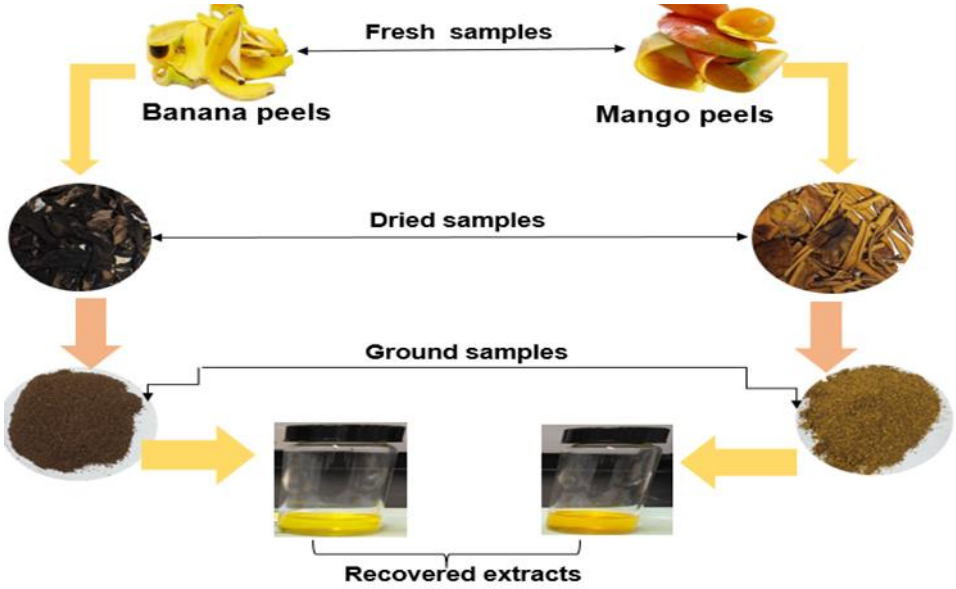

Figure 1: Samples preparation steps.

2.2 SFE Experimental Procedure

The two samples were independently extracted using supercritical fluid CO_2 extraction following a procedure reported by Chai et al., [5], with slight modification. The SFE set-up comprises a CO_2 supply tank connected to a high-pressure pump, supplying chilled CO_2 (99.9%) to the oven through a coil that heats the sample to the desired operating temperature. An automated back pressure regulator controls the flow and pressure in the extraction unit of the SFE experimental setup. A 5.0 g of each milled peel sample was loaded into the extraction vessel (Jasco, EV-3-50-2), while a 5% ethanol was used as a co-solvent (modifier). Extraction parameters such as temperature (40, 50, 60, 70, and 80℃, assumed the same as oven temperature), fixed CO_2 flow rate (9.8 g/min), and 25 MPa pressure were used. The extraction time was varied after the initial set pressure and the first

released purge from the automated back pressure regulator were achieved and attained respectively. Finally, the extract was collected after 30, 60, 90, 120, and 150 min in an amber glass flask immersed in an ice bath at atmospheric pressure. The extracts collected for the banana and mango peel samples were diluted with 2mL of ethyl acetate, to enhance the solubility of the recovered liquid extracts. The recovering solvent was carefully chosen considering its low polarity, compared with co-solvent (ethanol). Subsequently, the samples were stored in a refrigerator at 4℃. The percentage yield (X_o) of the recovered liquid extracts for the two samples were calculated using equation (1) by considering the dry basis, using the ratio of the weight of the extract (W_{extract}) to the weight of the milled sample used for extraction ($W_{\text{milled sample}}$).

$$X_0(\%) = \frac{W_{\text{extract}}}{W_{\text{milled sample}}} \text{ x } 100 \quad (1)$$

2.3 Experimental Procedure for the HPLC Analysis

The quantification of the bioactive compounds present in the respective recovered extracts and their corresponding external standards was carried out using SHIMADZU 20 AD HPLC profiling, integrated with an ultraviolet/diode array detector (UV/DAD). A reversed-phase (*RP*) separation was performed using a poroshell 120 EC-C18 (4.6 mm × 100 mm, 2.7 µm) column. The RP-HPLC analysis method reported by Krstonošia et al., [7] was adopted and modified such that the mobile phase composition used for the identification of the polyphenols (quercetin & gallic acid) was acetonitrile/acetic acid (98:2, v/v) with a pH of 5.3. At the same time, that of carotenoids (*β*-carotene), was methanol/trichloromethane (98:2, v/v) with a pH of 4.7 [8]. The prepared mixture solvents of the mobile phases were filtered using Whatman filter paper and degassed by a digital ultrasonic bath (Branson 8510) for thirty (30) min to remove air bubbles. The sample concentration was reduced by diluting with the respective mobile phase solvents using a 1:9 ratio, v/v.

Subsequently, 3.0 mL was measured and filtered using a 0.45 µm syringe filter (PTFE, hydrophobic, 25 mm outer ring). The injection volume was 20 µL for each sample and its corresponding prepared standard concentrations, while 40℃ was used as the column temperature. The flow rate was maintained at 1.0 mL/min, and the eluted samples were detected at 280 nm by the UV detector for the quercetin and gallic acid and 454 nm for the beta-carotene, respectively. All determinations were carried out in triplicates. The calibration curves were constructed by plotting a peak area (µV.s) graph on the *Y*-axis against concentrations (ppm) of the external standard solutions on the *X*-axis. This was used to quantify the amount of the target bioactive compounds and the data generated from the *RP*-HPLC analysis comprising the standard curve equation of each standard, the coefficient of determination (R^2), and the calculated quantity of bioactive compounds present in each sample extract expressed in milligrams per gram (mg/g).

3. Results and Discussions

3.1 Percentage Extract Yield (X_o)

The results of the percentage for the liquid extract recovered using SFE performed at temperatures from 40 – 80℃ over varied extraction times of 30 - 150 min was presented in Figure 2. The lowest yields were observed at the beginning of the experiment (30 min), while the highest was obtained after prolonged extraction (150 min). However, the increase in X_o proportionally with temperature is because of solubility improvement and faster diffusion caused by the temperature rise [9]. On the other hand, longer extraction times afforded sufficient interaction between extractable compounds and extractants, resulting in a higher extraction yield. The recovered X_o ranges from 2.61 - 3.95%, 0.62 - 1.58%, and 0.84 - 1.97% for palm fruit, banana, and mango peels, respectively. Accordingly, the palm fruit peel sample presented the highest yield followed by mango and banana peels. However, the lowest and highest quantity of the recovered liquid extract for palm fruit was 2.61% and 3.95% for the extraction conditions of 30 mins at 40℃ and 150 minutes at 80℃, respectively. As the temperature and extraction time increased, the amount of the recovered yield similarly increased. This suggests that the SFE rate of extract recovery for the milled palm fruit peels obtained using SFE is a forward reaction as relates to temperature and extraction time [10]. Therefore, this could be linked to the strong interaction between

the non-polar carotenoids in the palm fruit peel and the extractant (CO_2) solvent, following the principle that "like dissolves like".

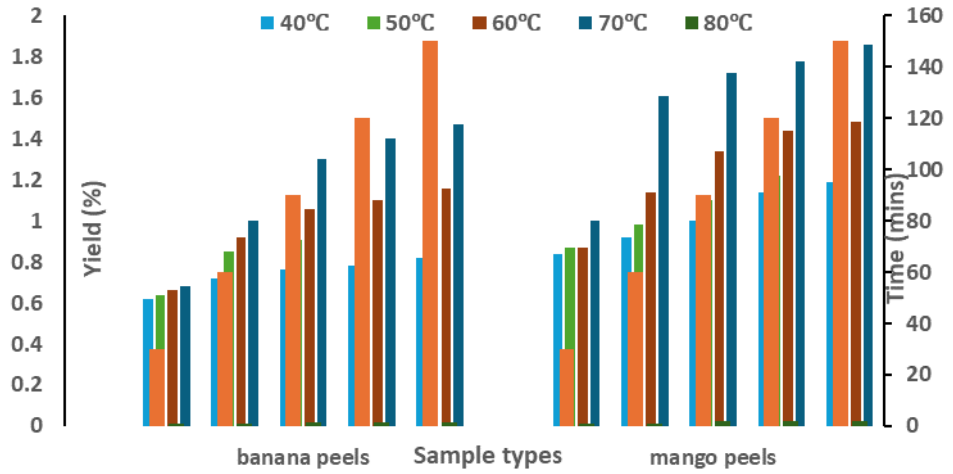

Figure 2: Result of extract yields obtained over time at different temperatures.

For the banana peel extract, the lowest yield of 0.62% was achieved at 40°C after 30 min while the largest yield of 1.58% was found after 150 min at 80°C. This result aligns with previous findings reported by Comim et al. [11], in which the authors recovered yields between 0.5 and 6.5% under similar conditions for the same sample type. However, reducing the extraction time and temperature to 10 min and 40°C respectively, led to a decrease in yield by 6.94% according to the findings reported by Anal et al., [12]. This indicates that prolonged extraction time at a higher temperature led to an improved yield due to greater diffusivity, enhanced mass transfer, and solubilization. However, the lower yields (1 – 10 %) recorded using SFE-derived extracts compared to conventional methods may be attributed to the selectivity of the extractant employed in which the non-overlap binding polarities between the interacting species hinder efficient solubilization giving rise to low recoveries [13].

On the other hand, the quantity of extracts recovered at 40°C after 30 min and 80°C after 150 min were 0.84 and 1.97, respectively for the milled mango peel sample. These results are within the same range of 0.50 - 3.8% reported by Souza et al., [14], Fernández et al., [15], and Santos et al., [16] for the same sample type, similar experimental conditions and extraction method respectively. In a similar study, Garcia-Mendoza et al., [17] and Guandalini et al., [18], recovered higher yields of 3.15 and 4.48% using SFE at 40°C and 30 MPa for 7.5 h, and 50°C and 25 MPa for 2 h respectively. The increased extract yield in their study could be due to the longer extraction time, which allowed for more efficient interaction between the extracting solvent and extractable solutes, giving rise to a higher diffusion rate [15-18]. Furthermore, an average yield of 0.8% was recovered from milled mango peels using SFE according to the findings of Gülçin, [19]. This was comparably lower than the 0.84% recovered in this work using the same sample type under similar extraction conditions. Overall, the inability of nonpolar CO2 to fully dissolve polar polyphenolic compounds in banana and mango peels hindered interaction and led to a lower yield recovery. However, the use of solvents with high polarity such as acetone and methanol can be considered suitable for extracting most phenolic compounds due to their greater hydrogen bonding capabilities that allow them to readily interact with other molecules and facilitate the dissolution of polar compounds. Consequently, this promotes greater interaction among the reacting compounds because of their high nucleophilic character [13].

3.2 Identification and quantification of bioactive compounds by HPLC

The presence of the bioactive compounds in palm fruit, banana, and mango peel extracts obtained following SFE was confirmed and identified by comparing their retention times and UV spectra with those of their respective external standard compounds. Comparatively, the chromatographic peaks and the retention times of the various analytes and those of their corresponding external standards were in close range and similar (Table 1). A clear and distinct separation of the compounds, with sharp chromatograms, was credited to the uniqueness

of the bonded phases and superficially porous particles of the poroshell column, thereby contributing to good baseline resolutions [20]. Similarly, the fast elution with shorter retention times (2-8 mins) of the polyphenolic compounds compared to the carotenoids could be attributed to the differences in polarity and mobile phase type used for the separation. Table 1, present the estimated quantity of quercetin in the banana extract was appreciably higher (4.6841 mg/g) than those of gallic acid and beta-carotene. The measured quantity of quercetin from the banana peel extract obtained in this work lies within the range of 0.6 - 6.9 ± 0.3 mg/g, reported by Comim et al., [12] using a similar quantification procedure. Similarly, the quantified amount of gallic acid obtained in this work (0.0489 mg/g) from banana peel extract was higher than 0.01519 and 0.0368 mg/g reported by Kandasamy and Aradhya [21] from banana peel extracts of acetone and methanol as respectively. Also, Kanta et al. [22] reported 0.0226 mg/g of gallic acid quantified from banana peel extract derived from solvent extraction using 95% ethanol. This quantity was lower than the 0.0489 mg/g obtained in this work. However, the variance in the recovered amount of gallic acid could be due to the differences in the cultivar types, extraction techniques, and other preparatory steps such as drying conditions and etc.

In another work, Sidhu and Zafar [23] reported that banana peel extracts are a rich source of polyphenolic compounds (0.044 – 0.851 mg/g), with many being flavonoids. However, the quantified amount of gallic acid in the mango peel extract (2.0254 mg/g) reported by Sidhu and Zafar [23], was higher and lower than 1.2983 mg/g and 3.6987 mg/g for quercetin and beta-carotene respectively obtained in this work as shown in Table 1 Comparatively, a relatively higher quantity of beta-carotene detected in this work, agreed with the results obtained in related findings for mango peel extract reported by Coelho et al., [24] using maceration. Moreover, the authors also established that the quantity of quercetin recovered from mango peel extracts of Tommy (0.3433 mg/g) and Atkins (0.3487 mg/g) species, were higher than the corresponding quantity of gallic acid (0.043 – 0.062 mg/g) quantified from the same mango cultivar. On the contrary, López-Cobo et al., [25]; and Ajila et al., [26] reported a higher amount of gallic acid from mango peel extract than quercetin using ultrasonic-assisted extraction and maceration respectively. These disparities could be linked to the differences in the mango species' variety and other operating conditions such as drying time and temperature, extraction, and quantification methods adopted. Sánchez-Camargo et al., [27] quantified beta-carotene content in mango peel extracts obtained using SFE as 0.0293 mg/g. This quantity was lower than the 3.6987 mg/g obtained in this work. Therefore, this variation could be due to differences in the experimental conditions such as UV detection range, mobile phase used, and injection volume [28].

Table 1: Quantity of bioactive compounds estimated (mg/g) in the three sample extracts by RP-HPLC–DAD

External standards	Fruit peel samples	Retention time (min)	Peak area	Standard equation	R^2	Estimated quantity (mg/g)	Quantity (mg/g) reported by other authors
Quercetin	-	2.563	16200537	$y = 16557x - 2x10^6$	0.9754	-	-
	Banana	2.565	6617230	-	-	4.6841	6.9*
	Mango	2.563	388499	-	-	1.2983	NQ
Gallic acid	-	2.459	2772466	$y = 45815x - 54305$	0.9955	-	-
	Banana	2.581	194499	-	-	0.0489	0.036**
	Mango	2.455	75006	-	-	2.0254	NQ
β-carotene	-	53.40	625881	$y = 633.04x - 43379$	0.9662	-	-
	Banana	54.32	37293	-	-	1.1469	NQ
	Mango	54.26	216777	-	-	3.6987	1.9****

Key: NQ = not quantified, ‘’-‘’ = not applicable, * = [29], ** = [30], *** = [31] and **** = [32].

Note: The recovered liquid extracts used for the quantification of the target compounds were obtained using SFE experimental conditions of 25MPa pressure, a CO2 flow rate of 9.8 g/min, and 60℃ after extraction time of 90 min using 5.0 g of each sample.

4. Conclusion

SFE successfully recovered liquid extracts of banana, and mango peels, with the highest quantity obtained at 80℃ temperature and 150 mins extraction time. The extraction was viable at various temperatures of 40, 50, 60, 70, and 80℃, however, longer extraction time allows adequate interaction between extractant and compounds, resulting in maximum extract yield and this was considered the reason for recovering higher extract yield at elongated extraction times. Also, the presence of the target compounds namely quercetin, gallic acid, and β-carotene in the recovered liquid extracts was confirmed and quantified using RP-HPLC and the validity of the technique was appraised considering the high R2 values (greater than 0.96) indicating a good correlation. Finally, it is recommended that using suitable green co-solvents (water) or corresponding blends can improve extraction efficacy by matching the solubility of target compounds and conducting multiple extraction cycles, using optimized SFE condition parameters like pressure, temperature, and flow rate can also maximize yield while maintaining product quality.

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