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EFFECTS OF DRYING TECHNIQUES ON PHENOLICS CONTENT AND ANTIOXIDANT ACTIVITY OF WATER-SOLUBLE PALM FRUIT EXTRACT

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ABSTRACT

Water-soluble palm fruit extract (WSPFE) is produced from the vegetation liquor of palm oil milling. It comprises water-soluble phenolic acids including protocatechuic acid (PCA), p*-hydroxybenzoic acid (*p*-HBA) and three isomers of caffeoyl-shikimic acid (3-O-CSA, 4-O-CSA and 5-O-CSA). WSPFE has been shown to exhibit a range of bioactive properties both* in vitro *and* in vivo*. The present study investigated the effects of freeze and spray drying, on the phenolic content, antioxidant capacity, organoleptic properties, hygroscopicity and moisture content of WSPFE. To quantify the concentrations of PCA,* p*-HBA and 3-O-CSA within WSPFE, an isocratic HPLC-UV method was developed and validated. The HPLC method employed a mobile phase system consisting of 20% methanol and 80% water, with a flow rate of 0.8 mL min^{-1}, a running time of 40 min and UV detection at 280 nm. Comparative analysis revealed that freeze drying maintained a marginally higher retention of phenolic compounds. Spray drying resulted in WSPFE with lower hygroscopicity and moisture content. Spray drying showed a slightly higher concentration of PCA (23 ± 0.3 µg mL^{-1}) compared to freeze drying (22 ± 0.1 µg mL^{-1}). No significant differences were found in the concentrations of* p*-HBA acid and 3-O-CSA between freeze drying and spray drying.*

Keywords: HPLC-UV, phenolics, quantification, validation, water-soluble palm fruit extract.

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INTRODUCTION

Palm oil is extracted from the pulp of the fruit of *Elaeis guineensis*, a tropical monocotyledon tree (Sundram et al., 2003). During the palm oil extraction process, large volumes of vegetation liquor are produced as a by-product which contains valuable hydrophilic phenolics (Sambanthamurthi et al., 2011).

The Malaysian Palm Oil Board (MPOB) has implemented environmentally friendly techniques, without the use of solvents, to recover water-soluble palm fruit extract (WSPFE) from this vegetation liquor of palm oil production. WSPFE has exhibited numerous *in vitro* and *in vivo* bioactive effects. These effects include anti-diabetic, anti-cancer, anti-hypertensive, anti-obesity, cardioprotective and neuroprotective properties (Leow et al., 2013, 2022a). Moreover, phase I clinical trials conducted on healthy subjects have confirmed the safety of WSPFE (Fairus et al., 2018). The primary bioactive components of WSPFE are phenolic acids, namely protocatechuic acid (PCA), *p*-hydroxybenzoic acid (*p*-HBA) and three distinct isomers of caffeoylshikimic acid (3-O-CSA, 4-O-CSA and 5-O-CSA) (Leow et al., 2022a). Potential health benefits of WSPFE could be attributed to its phenolic content (Goleniowski et al., 2013; Sambanthamurthi et al., 2011; Saxena et al., 2012; Syarifah-Noratiqah et al., 2019).

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After the extraction procedures, plant extracts are usually dried using different techniques to remove the extraction solvents and to get a dry mass of the plant extract. The drying process is also performed to enhance the shelf life and stability of the plant extracts. However, the quality and quantity of the dried plant extracts are influenced by the drying technique and conditions. The drying process could also affect the concentration and activity of bioactive compounds present in the plant extracts (Belwal et al., 2022; Nakbanpote et al., 2019). In addition, the drying process might affect the physicochemical and organoleptic properties of the dry products. Thus, it is necessary to optimise the drying conditions for each plant extract. Energy sources, physical properties of the product, duration of the drying process and the purpose of the drying should be considered before choosing the optimum drying method (Langford et al., 2018).

Plant extracts could be dried by different techniques such as spray drying, freeze drying, oven drying, vacuum drying, fluid bed drying and sun-drying (ElNaker et al., 2021; Singh et al., 2013). Spray drying is a widely used method to dry plant extracts and natural products, particularly in the food, nutraceutical and pharmaceutical industries. It is a simple, fast and cost-effective technique that is ideal for large-scale productions (Gallo et al., 2015). On the other hand, freeze drying, also known as lyophilisation, is a common drying technique for plant extracts that contain thermolabile bioactive compounds. This method operates at low temperatures, making it suitable for such compounds. Before drying, the extract solution should be frozen and aqueous. During freeze drying, the frozen product is dehydrated under a vacuum sublimation process (Belwal et al., 2022; Pudziuvelyte et al., 2020).

The primary objective of this research was to determine the optimal drying method for WSPFE by conducting a comparative analysis of the phenolics content and properties of the extract after freeze-drying and spray-drying. Freeze-drying and spray-drying were selected as the preferred methods for drying WSPFE due to their ability to maintain the integrity of bioactive compounds and produce high product quality. Freeze drying involves sublimation at low temperatures which is suitable to dry heat-sensitive compounds. Meanwhile, spray drying produces a fine, uniform powder with excellent flowability and minimal thermal degradation of compounds (Shishir & Chen, 2017; Sollohub & Cal, 2010). These techniques are studied over traditional methods such as hot air drying, which may compromise bioactive compounds due to high temperatures (Hii et al., 2009), or vacuum and microwave drying, which can result in uneven drying and potential loss of volatile substances (Mujumdar, 2014; Zhang et al., 2006). Freeze and spray-dried WSPFE were investigated previously to evaluate their potential anti-diabetic mechanism (Leow et al., 2022b). However, these two drying methods were not compared regarding their ability to retain bioactive phenolic compounds. The current study aims to fill this gap by evaluating and comparing the effects of freeze drying and spray drying on the total phenolic content, major phenolic constituents, product quality and antioxidant properties of WSPFE.

Considering the potential development of WSPFE into nutraceutical or pharmaceutical products, accurate quantification of phenolic acids is important for ensuring product quality and efficacy. High-performance liquid chromatography (HPLC) is a widely used method for phenolic separation and quantification due to its high resolution and sensitivity (Çayan et al., 2020; Kalili & De Villiers, 2011; Spáčil et al., 2008). HPLC analysis of WSPFE was previously carried out using a gradient HPLC method which involved methanol, acetonitrile and sodium sulphate as a mobile phase system and a running time of 60 min (Sambanthamurthi et al., 2011). For this reason, a novel and efficient isocratic HPLC-UV method was developed and validated to accurately quantify the concentrations of PCA, *p*-HBA and 3-O-caffeoylshikimic acid (3-O-CSA) in freeze and spray-dried WSPFE. Isocratic elution involves using a constant mobile phase composition throughout the entire process, which simplifies method development and operation. This consistency can result in more reliable retention times and peak areas, making it easier to attain consistent results across various runs and batches of samples (Snyder et al., 2011). The selection of *p*-HBA and 3-O-CSA was based on their high abundance in WSPFE, as indicated by previous studies (Sambanthamurthi et al., 2011). PCA, on the other hand, has demonstrated significant antioxidant properties even at low concentrations, along with various therapeutic effects documented in different studies (Kakkar & Bais, 2014; Semaming et al., 2015).

The method validation process was carried out according to the ICH Expert Working Group (2005) guidelines for analytical procedures including system suitability, accuracy, specificity, precision, robustness, linearity, limits of detection (LOD) and limits of quantification (LOQ) (Branch, 2005). This proposed method provides a simple, cost-effective and practical approach for detecting phenolic compounds with minimal usage of organic solvents and acids. To the best of our knowledge, no previous isocratic reversed-phase HPLC (RP-HPLC) method has been presented for measuring phenolics in WSPFE. Besides, the impact of freeze and spray drying techniques on the quality and quantity of WSPFE was not reported previously.

MATERIALS AND METHODS

Materials and Chemicals

Analytical standards of *p*-HBA and PCA were obtained from Thermo Fisher Scientific (USA), while 3-O-CSA was obtained from ChemFaces (China). Methanol (HPLC grade), dimethyl sulphoxide (DMSO), and formic acid were procured from Thermo Fisher Scientific (USA). The WSPFE samples were provided by MPOB in liquid form and were frozen and spray-dried before analyses.

Freeze Drying of WSPFE

WSPFE solution (200 mL) was passed through a 0.45 µm filter paper and collected in a container that was weighed beforehand. The collected solution was then frozen at -20°C for two days. After that, the frozen sample was lyophilised using a freeze dryer (Labconco, USA) at -50°C for three days. The vacuum pressure was kept at 0.06 mBar during the process. The dried sample was collected from the drying vessel and kept in a freezer before the analysis procedures.

Spray Drying of WSPFE

The process of spray drying WSPFE involved using a mini-spray dryer (Büchi B-290, Büchi, Flawil, Switzerland). A 200 mL solution of WSPFE was first filtered using a 0.45 µm filter paper and then used as a feeding solution for spray drying. The feeding solution was fed into the drying chamber at a rate of 2 mL min^{-1} while maintaining an inlet temperature of 85°C and an outlet temperature of 75°C. The aspirator value was kept constant at 100%. Dry WSPFE was obtained from the collection vessel and kept in a desiccator before analyses.

Instrumentation and Chromatographic Conditions

The chromatographic analysis was conducted using a Shimadzu Prominence HPLC System (Shimadzu Corporation, Kyoto, Japan), which includes a dual pump system (LC-20AD), an autosampler, a column oven, an on-line degasser and a UV-Vis detector. Data processing and analysis were performed using the Shimadzu Lab-Solution software. Chromatographic separation of the phenolics was achieved on an RP-HPLC C-18 ODS column (200 x 4.6 mm) (Thermo Fisher Scientific, USA), maintained at 25°C. The flow rate was set at 0.8 mL min^{-1}, and the injection volume was 20 µL with UV detection occurring at 280 nm. The mobile phase consisted of a mixture of 80:20 deionised (DI) water and methanol (v/v), with 0.1% formic acid added to adjust the pH to 2.5 in the aqueous phase. Prior to use, mobile phase solutions were filtered through a 0.45 µm Millipore filter and degassed by ultrasonication for 20 min. The total runtime for each chromatographic run, for both the standard solutions and WSPFE samples were 40 min.

Standards and Sample Preparation

Stock solutions of the analytical standards were prepared by dissolving 1 mg of *p*-HBA and PCA in 1 mL methanol and 1 mg of 3-O-CSA in 1 mL dimethyl sulfoxide (DMSO). Solutions of phenolics were diluted using a 1:1 v/v ratio of deionised water and methanol. A series of dilutions were then made to produce phenolic standard solutions at various concentrations, specifically 6.25, 12.50, 25.00, 50.00 and 100.00 µg mL^{-1}. Samples of WSPFE, both freeze-dried and spray-dried, were dissolved in deionised water to achieve a concentration of 5 mg mL^{-1}. These solutions were subsequently filtered through a 45 µm filter prior to being injected. Three injections were carried out for each WSPFE sample, and the average peak absorbance was recorded.

Method Validation

The specificity of the method was assessed by subjecting the WSPFE solution to both acid and alkaline hydrolyses using 0.1 M hydrochloric acid (HCl) and 0.1 M sodium hydroxide (NaOH), respectively. Additionally, various parameters were monitored to confirm the system's suitability for the intended analysis, including the tailing factor of the peaks, the number of theoretical plates (NTP) and the resolution between peaks. An average of twelve replicates of each phenolic standard solution was used to measure system suitability. Linearity was established using three replicates across six concentrations of a standard mixture of phenolic acids (*p*-HBA, PCA, 3-O-CSA). Calibration curves were created by plotting the ratio of peak area against the concentrations of the phenolics (6.25, 12.50, 25.00, 50.00, 100.00 µg mL^{-1}). Linear regression analysis was performed using Microsoft Excel to determine the intercept, slope and correlation coefficient (r^2) for each phenolic compound. Standard solutions were prepared within the concentration range observed in WSPFE samples. The LOD and LOQ were calculated based on the signal-to-noise (S:N) ratio of the lowest concentration samples, with LOD expected to produce a S:N ratio of 3-10, while LOQ should fall within a S:N ratio of 10-20.

The precision of the developed method was evaluated both intra-day and inter-day for three phenolic standard solutions at different concentrations. For intra-day precision, the relative standard deviation (RSD%) was measured across three replicates at three concentrations (6.25, 25.00, 100.00 µg mL^{-1}) within a single day, resulting in nine total measurements, in accordance with ICH

guidelines for the development of analytical methods (Kazusaki et al., 2012). To assess inter-day precision, the three concentrations of the phenolics (6.25, 25.00, 100.00 µg mL^{-1}) were tested three times per day over four days. The accuracy of the method was verified by calculating the average recovery of phenolic acid standards that were deliberately added to the WSPFE sample at the same three concentrations. Furthermore, accuracy was also tested by spiking these concentrations into distilled water to serve as a blank sample. Three replicates were injected for each concentration. The recovery of phenolics was calculated using the following Equation (1).

$$\text{Recovery} = \frac{\text{Conc. spiked sample - Conc. unspiked sample}}{\text{Conc. of added standard}} \times 100 \quad (1)$$

Note: Conc. – concentration.

The robustness of the method was tested through slight alterations in the methodological conditions. The robustness was evaluated by analysing three different concentrations of the phenolic solutions using altered mobile phase ratios of 22:78 and 18:82 methanol to water (v/v), and by adjusting the pH of the mobile phase from the standard 2.5 to both 2.3 and 2.7. Additionally, the experiments were conducted at temperatures of 23°C and 27°C, deviating from 25°C. The peak responses under these varied conditions were recorded and compared with those obtained under the standard conditions to assess any impacts.

Organoleptic Properties of Freeze and Spray-Dried WSPFE

The sensory attributes including taste, colour, odour and appearance of the dried WSPFE samples were observed and recorded. Taste and odour were tested by human sensory evaluation using tasting by tongue and smelling.

Antioxidant Effects of Freeze and Spray-Dried WSPFE

The antioxidant properties of the dry WSPFE samples were evaluated using the DPPH free radical scavenging method. To prepare the stock solution, 2.4 mg of DPPH was dissolved in 10.00 mL of methanol. This stock was then diluted with an additional 45.00 mL of methanol to create the working solution. A sample of the extract, 0.15 mL, was mixed with 2.85 mL of the working solution. Methanol served as both the blank and the negative control, while ascorbic acid (vitamin C) was employed as the positive control. The samples were placed in a 96-well plate and incubated in the dark at room temperature for 1 hr. Absorbance readings were taken at a wavelength of 515 nm using a UV-vis microplate reader, with six replicates recorded for each sample. The inhibition of DPPH was calculated using the following Equation (2).

$$\frac{\text{(Abs control - Abs sample)}}{\text{(Abs control)}} \times 100 \quad (2)$$

Note: Abs - absorbance.

Phenolic Content of Freeze and Spray Dried WSPFE

The total phenolic content of the dry WSPFE samples was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965). For each analysis, 100 µL of sample was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.00 mL of distilled water. After allowing the mixture to stand for 5 min, 1.5 mL of 7.5% sodium carbonate solution was added. The resulting mixture was then left to incubate for 2 hr in the dark at room temperature before measurement. Each sample was tested in six replicates. A calibration curve was established using serial dilutions of gallic acid (0.50, 0.25, 0.10, 0.050, 0.025 mg mL^{-1}). Methanol served as the blank for the experiments. Absorbance was measured at 765 nm using a UV-VIS microplate reader. Total phenolic content (TPC) was calculated following the Equation (3).

$$TPC = \frac{C \times V}{M} \quad (3)$$

where, C is the calculated concentration from the calibration curve, V is the volume used in mL and M is the extract weight in g.

Hygroscopicity of Freeze and Spray-Dried WSPFE

Hygroscopicity was assessed using a method outlined in the European Pharmacopoeia (Council of Europe, 2013). Initially, an empty glass vessel was weighed. Subsequently, a dry WSPFE sample was added to this vessel, which was then weighed again. The vessel containing the sample was placed in a stability chamber (Clean Tech, Malaysia) maintained at 25 ± 1°C and 80 ± 2% relative humidity. After 24 hr in the stability chamber, the vessel was weighed once more. The percentage increase in mass was calculated using the Equation (4).

$$\text{Hygroscopicity} = \frac{m3 - m2}{m2 - m1} \times 100 \quad (4)$$

where, $m1$ represents the weight of the empty glass vessel, $m2$ is the combined weight of the glass vessel and the sample at the start, and $m3$ is the combined weight after exposure to humidity for 24 hr.

Drying-induced Loss of Freeze and Spray Dried WSPFE

Moisture content was evaluated through the loss on drying method. A gram of the dry WSPFE samples was placed into a glass container that had been pre-weighed. This container was then positioned inside an oven and maintained at 100°C for 3 hr. After heating, the sample was weighed again, and the moisture content was determined by calculating the decrease in weight of the sample.

RESULTS AND DISCUSSION

HPLC Method Development and Optimisation

Various mobile phase compositions and concentrations were tested to refine the separation of phenolic acids. Initial trials using acetonitrile and water, with and without additives such as formic acid and acetic acid, yielded poor peak shape and resolution. Subsequently, methanol was experimented in varying proportions of 20:80, 50:50 and 80:20 with water. The best separation and peak resolution, exceeding a resolution factor of 2, was achieved with a 20:80 methanol to water ratio, incorporating 0.1% formic acid at a pH of 2.5. Moreover, the optimal wavelength for detecting the phenolic acid standards was identified as 280 nm after testing at wavelengths ranging from 200-320 nm. A temperature setting of 25°C was selected to prevent potential thermal degradation of the phenolic acids. The most effective flow rate determined was 0.8 mL, maintaining a stable column pressure of 12.5 MPa and facilitating efficient separation. With an isocratic mobile phase system of 20% methanol and 80% water, this method represents a simple approach with reduced solvent consumption compared to the previously used gradient RP-HPLC method for WSPFE analysis. The only reported method for WSPFE analysis has used a gradient system composed of a mixture of methanol, acetonitrile and sodium sulphate with 60 min of running time (Leow et al., 2022a; Sambanthamurthi et al., 2011), while the currently developed method involves 40 min of running time. The isocratic system is reproducible with less complex equipment and does not require precise gradient mixing capabilities, which reduces the cost and maintenance (Habyalimana et al., 2017; Snyder et al., 2011).

As shown in *Figure 1*, PCA eluted first with a retention time between 6.9 and 7.0 min, followed by *p*-HBA at 11.5 min, and 3-O-CSA at 26.6 min. The separation and elution order of the three phenolics aligns with that of the previously reported method. The intensity of the PCA peak in the WSPFE sample is low which is due to the low concentration of the compound in the extract compared to *p*-HBA and 3-O-CSA (Sambanthamurthi et al., 2011). PCA eluted earlier than *p*-HBA, possibly due to higher polarity and less hydrophobicity (Robbins, 2003), while 3-O-CSA, a more complex compound, eluted at the end likely due to higher molecular size and more possible interactions with the stationary phase.

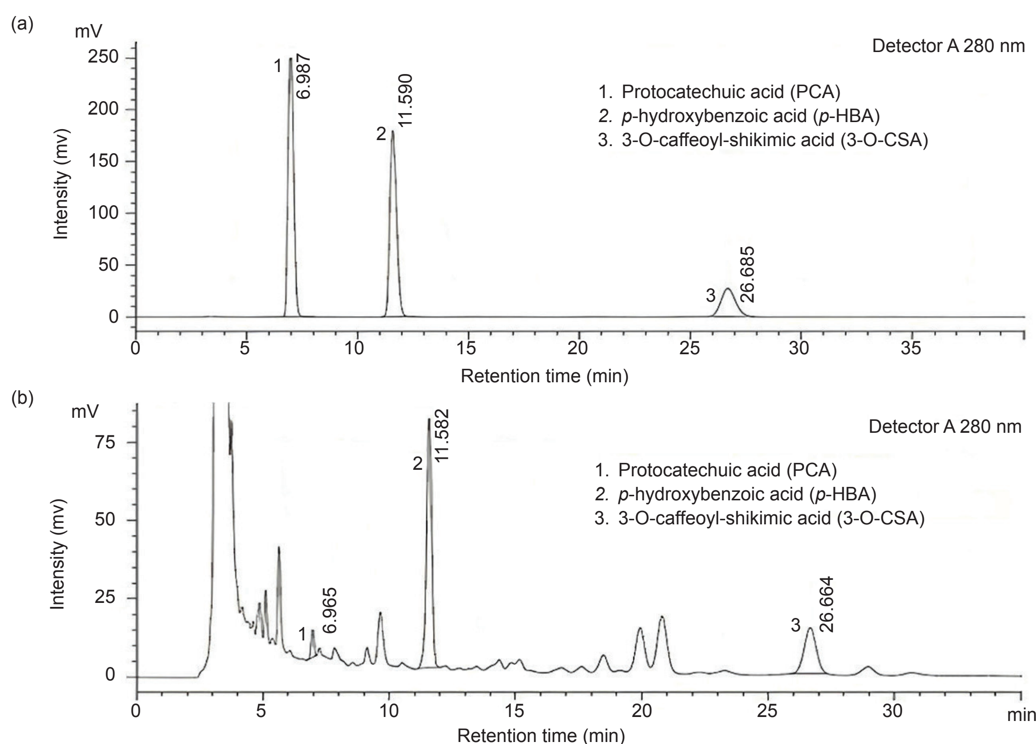

Figure 1. Chromatograms of PCA, p-*HBA and 3-O-CSA in (a) standard solutions, and (b) WSPFE sample at 280 nm.*

Method Validation

Specificity and system suitability. Specificity is described as the capacity of an analytical method to distinguish and measure compounds distinctly in the presence of other potential analytes or impurities (Branch, 2005). This method's specificity was proven by successfully separating and distinguishing the peaks in the standard solution and WSPFE sample despite the existence of other compounds. Additionally, after undergoing acid and alkaline hydrolyses using hydrochloric acid and sodium hydroxide, PCA, *p*-HBA and 3-O-CSA were separated and detected clearly, further confirming the method's specificity (*Figure 2*). System suitability tests were performed to confirm the effectiveness of the equipment, electronics and the designated analytical method under specified conditions (Aminu et al., 2019; Shabir, 2003). *Table 1* presents the system suitability parameters for the three phenolic compounds. All compounds met the recommended criteria with a resolution greater than 2, the number of theoretical plates (NTP) exceeding 2,000 and a tailing factor less than 2.

Linearity, calibration curves and sensitivity. Linearity indicates a proportional response between the concentration of analytes and their analytical signals (Kazusaki et al., 2012). The correlation coefficient (r^2) for each phenolic standard was derived from the regression lines of their calibration curves. These curves were established by plotting concentrations ranging from 6.25-100.00 µg mL^{-1} against the mean peak areas. *Figure 3* illustrates the calibration curves for each phenolic compound. *Table 2* confirms that the correlation coefficients for all three compounds were ≥0.999, demonstrating robust linearity within the tested range. LOD, represents the smallest concentration of an analyte detectable by a specific method, whereas LOQ, is the minimum concentration at which an analyte can be reliably quantified with suitable precision and accuracy (Aminu et al., 2019; Shabir, 2003). *Table 2* reveals that the LODs for PCA, *p*-HBA and 3-O-CSA were 0.16, 0.22 and 1.65 µg mL^{-1} respectively, while the LOQs were 0.48, 0.65 and 5.00 µg mL^{-1}. These results confirm the method's sensitivity for detecting and quantifying these phenolics at low concentrations.

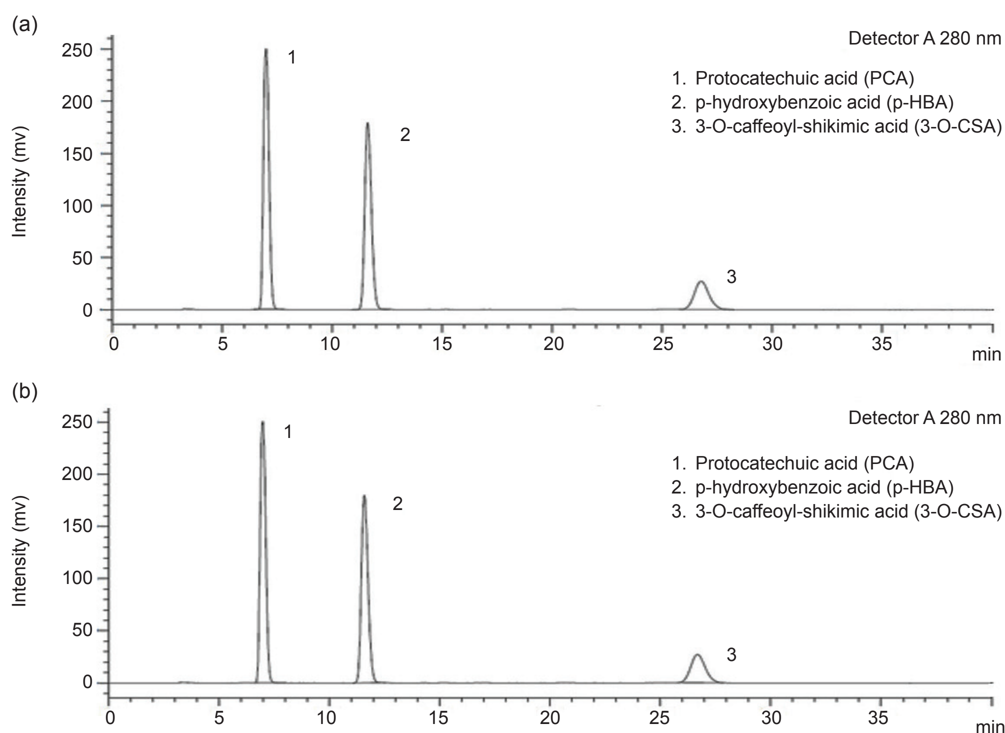

Figure 2. Chromatograms of standard solutions containing (1) PCA, (2) p*-HBA and (3) 3-O-CSA after (a) acid, and (b) alkaline treatments.*

TABLE 1. SYSTEM SUITABILITY PARAMETERS

Parameters	PCA (n=12)	*p*-HBA (n=12)	3-O-CSA (n=12)	Recommended values (Shabir, 2003)
Resolution	>2	>2	>2	>2
NTP	3 680	6 928	7 849	>2 000
Tailing factor	1.154	1.19725	1.116	≤2

Note: NTP - Number of theoretical plates.

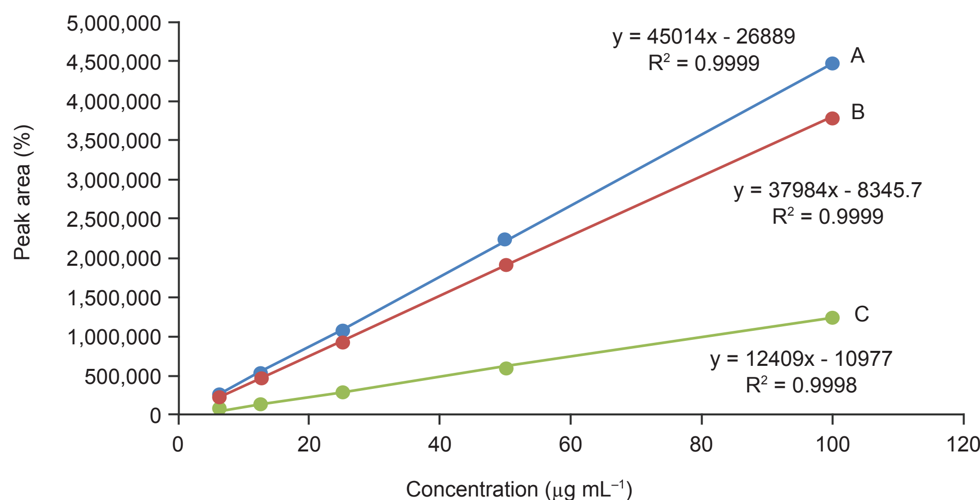

Figure 3. Calibration curves for the phenolic standards (a) PCA, (b) p-HBA, *and (c) 3-O-CSA (peak area vs. concentration in µg mL^{-1}).*

Precision and accuracy. Accuracy in an analytical method refers to how closely the measured values align with the true or known values (Kazusaki et al., 2012). The method's accuracy was evaluated by the percentage recovery of phenolic standards that were added to the WSPFE samples at concentrations of 6.25, 25.00 and 100.00 µg mL^{-1} (diluted 1:5). The concentration of the recovered phenolics was determined using the average peak area from three measurements for each concentration. The recovery rates for all standards were within 100 ± 5%, reflecting the high accuracy of the method. Additionally, the repeatability (intra-day) and intermediate precision (inter-day) were assessed using the mean relative standard deviation (RSD%), which remained below 2% across various concentrations for all phenolic standards, demonstrating the reliable precision of the method (Shabir, 2003). *Table 3* illustrates the accuracy and precision data for the three phenolics at different concentrations.

Robustness. Robustness was evaluated to determine the method's resilience to minor intentional variations in experimental conditions (Kazusaki et al., 2012; Shabir, 2003). Robustness was tested by making slight adjustments to the mobile phase composition, pH and column temperature for three different concentrations of standard solutions (6.25, 25.00 and 100.00 µg mL^{-1}). The peak responses for the three phenolic compounds PCA, *p*-HBA and 3-O-CSA, remained stable and the relative standard deviations (RSD%) were under 2% for all tested concentrations, indicating the method's robustness.

Quantity of the Main Phenolics in Freeze and Spray Dried WSPFE

Spray drying is a process that involves atomising a liquid feed into fine droplets and quickly drying them with hot air, resulting in the formation of a powder. This method is efficient in producing particles of uniform size. Freeze

TABLE 2. LINEARITY EQUATION, CORRELATION COEFFICIENT, LOD AND LOQ FOR THE PHENOLICS IN WSPFE

Compound	Linearity equation (6.25 – 100.00 µg mL^{-1})	r^2	LOQ (µg mL^{-1})	LOD (µg mL^{-1})
PCA	y = 45014x – 26889	0.9999	0.48	0.16
p-HBA	y = 37984x – 8345.7	0.9999	0.65	0.22
3-O-CSA	y = 12409x – 10977	0.9998	5.00	1.65

Note: LOQ - limits of quantification; LOD - limit of detection.

TABLE 3. MEAN RECOVERY (ACCURACY), INTRA- AND INTER-DAY PRECISIONS OF THE THREE PHENOLIC ACIDS

Compound	Recovery % (n=3)			Intra-day precision (RSD%) (n=3)			Inter-days precision (RSD%) (n=12)		
	100	25	6.25	100	25	6.25	100	25	6.25
PCA	104.6	98.0	96.7	0.6	0.7	1.4	0.6	0.7	1.6
p-HBA	98.8	100.5	102.0	0.5	0.9	1.6	0.5	0.9	1.6
3-O-CSA	105.0	101.7	99.4	1.3	0.2	1.5	1.3	1.9	1.5

Note: RSD - Relative standard deviation (RSD%).

drying (lyophilisation) involves freezing the material and then reducing the pressure to allow the ice to sublimate directly from solid to gas, preserving the structure and activity of sensitive compounds (Pinto et al., 2021; Santos et al., 2017). As shown in *Table 4*, the concentration of PCA was significantly higher in spray-dried WSPFE. Although higher levels of *p*-HBA and 3-O-CSA were observed in freeze-dried WSPFE, the differences were insignificant. According to Chumroenphat et al. (2021), protocatechuic acid is slightly heat-sensitive, which might explain why it is not affected by the high temperatures of a spray dryer. On the other hand, *p*-HBA was identified as highly sensitive in the same study, which aligns with the result of the current study showing a higher concentration of the compound in the freeze-dried extract. The process of drying is fast in a spray dryer, and the exposure time to the hot air is very short (Shishir & Chen, 2017), which could be the reason for the minimal variation in the phenolic levels between the two methods.

Organoleptic Properties of Dry WSPFE

The effects of freeze drying and spray drying on the organoleptic properties of WSPFE were compared. *Table 5* shows that the taste and odour of the extract remained consistent before and after both drying processes. However, there were slight differences in colour post-drying. WSPFE contains a high amount of fruit sugars, which may account for the sweet taste of the dried extract, as reported by Ibrahim et al. (2020). Spray drying produced a dry sticky powder, whereas freeze drying resulted in a sticky, paste-like product. This stickiness can be attributed to the sugar content, which is hygroscopic and tends to absorb moisture from the environment (Feng et al., 2018). Higher moisture content was linked to a reduction in the glass transition temperature leading to sticky behaviour. Thus, the sticky, paste-like product of the freeze dryer is probably produced due to higher moisture content (Braga et al., 2019). Freeze dryers have been found to produce voluminous flakes which could not be turned or ground into flowable powder (Febriyenti et al., 2014). On the other hand, a spray dryer is well known for its ability to produce dry powder of plant extract. Gallo et al. (2015) explored spray drying effects on several aqueous extracts, which produced powder with low moisture content and good flow properties.

Antioxidant Effects and Phenolic Content of Dry WSPFE

The antioxidant capacity of dry WSPFE samples was assessed by their ability to scavenge free radicals, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) to estimate the antioxidant effects of both freeze-dried and spray-dried extracts. The DPPH assay, which is stable and cost-effective, measures either hydrogen atom transfer or single electron transfer. The antioxidant properties of WSPFE are linked to their capability to donate hydrogen atoms (Ibrahim et al., 2013; Sambanthamurthi et al., 2011), with the antioxidant potential of the phenolics being proportional to the hydroxyl groups in their structure. As shown in *Table 6*, the IC_{50} value for the freeze-dried WSPFE was slightly lower at 2.40 ± 0.15 mg mL^{-1} compared to 2.41 ± 0.07 mg mL^{-1} for the spray-dried extract, suggesting a marginally higher antioxidant capacity in the freeze-dried form. However, the difference in antioxidant activity between the two drying methods was minimal, at just 0.01 mg mL^{-1} and likely influenced by the phenolic content of the extracts.

To further understand the impact of drying methods on the phenolics, the Folin-Ciocalteu method was employed, using gallic acid for calibration. The phenolic content was slightly higher in the freeze-dried WSPFE at 42.10 ± 0.28 mg g^{-1} GAE compared to 41.49 ± 0.50 mg g^{-1} GAE in the spray-dried extract as illustrated in *Table 6*. Freeze drying tends to better preserve bioactive and thermolabile compounds in extracts (ElNaker et al.,

TABLE 4. CONCENTRATION OF MAIN PHENOLICS IN DRY WSPFE SAMPLES

Drying technique	PCA (µg mL^{-1})	*p*-HBA (µg mL^{-1})	3-O-CSA (µg mL^{-1})
Freeze drying	22 ± 0.1	300 ± 11.0	364 ± 4.6
Spray drying	23 ± 0.3*	298 ± 4.3	361 ± 2.6

Note: *- $p \leq 0.05$.

TABLE 5. ORGANOLEPTIC PROPERTIES OF FREEZE AND SPRAY-DRIED WSPFE

Drying technique	Taste	Odour	Colour	Appearance
Freeze-dried	Sweet	Caramelised	Dark brownish	Sticky paste-like product
Spray-dried	Sweet	Caramelised	Light brownish	Sticky powder

TABLE 6. ANTIOXIDANT EFFECTS, PHENOLIC CONTENTS, HYGROSCOPICITY AND MOISTURE CONTENT (± SEM) OF DRY WSPFE (N = 3)

Drying technique	DPPH IC_{50} (mg mL^{-1})	TPC (mg g^{-1} GAE)	Hygroscopicity	Moisture content (mg g^{-1})
Freeze-dried	2.40 ± 0.15	42.1 ± 0.3	14.7 ± 0.2	18.3 ± 6.0
Spray-dried	2.41 ± 0.07	41.5 ± 0.5	12.6 ± 0.8	15.2 ± 0.7

2021; Soares et al., 2012). Despite this, the variations in phenolic content post-drying were minor. Even with the higher temperatures involved in spray drying, significant phenolic loss did not occur, possibly due to the brief exposure of particles to the drying gas. Consequently, both drying techniques showed a nearly equivalent effect on the phenolic content of WSPFE.

Hygroscopicity and Loss on Drying of Dry WSPFE

Hygroscopicity is described as the capacity of materials to absorb and adsorb moisture from their surroundings. It influences various properties of materials such as stability, flow characteristics, compression and dissolution rates in pharmaceuticals. Thus, it is critical to assess the moisture absorption capabilities of drug substances (Allada, 2016; Arigo et al., 2019). The hygroscopic nature of WSPFE was evaluated using a standard procedure outlined in the European Pharmacopoeia. Compared to its spray-dried counterpart, the freeze-dried WSPFE exhibited greater hygroscopicity, indicating a stronger propensity to attract ambient moisture *Table 6*. This characteristic is likely due to the presence of sugars in WSPFE, which are known to absorb moisture (Feng et al., 2018; Muzaffar et al., 2015). It was reported that lower hygroscopicity of spray-dried extracts might be due to higher glass transition than the freeze-dried extract. The glass transition temperature (T_g) of a product has a significant impact on its hygroscopicity. Generally, higher T_g values are associated with lower hygroscopicity (Zhang et al., 2018). Research has shown that spray drying produces powders with a higher T_g compared to freeze drying, resulting in reduced hygroscopicity (Wang et al., 2020). Additionally, freeze-dried WSPFE demonstrated a higher moisture content, which can impact the physical characteristics of the product, such as its flowability and stickiness. In contrast, spray-dried extracts were found to possess lower levels of moisture and hygroscopicity (Feng et al., 2018; Wang et al., 2020). The lower moisture content of spray-dried extract could be due to the higher temperatures used during the drying process with a low feed flow rate. At lower flow rates, there is higher contact time between the feed and the drying air, resulting in efficient heat transfer and higher water evaporation (Tonon et al., 2008).

CONCLUSION

Following the ICH Expert Working Group (2005) guidelines, a new isocratic RP-HPLC method was developed and validated for the separation and quantification of three phenolic acids, namely PCA, *p*-HBA and 3-O-CSA in WSPFE. The proposed method has the advantage of using a low composition of organic solvents, which makes it cost-effective and more environmentally friendly. In addition, the sample preparation process was simple and rapid. The developed method was validated via several tests. It demonstrated acceptable ranges of specificity, linearity, accuracy, precision and robustness. WSPFE was subjected to both spray-drying and freeze-drying methods. The freeze-drying process resulted in a paste-like, sticky form of WSPFE, while spray drying produced a sticky powder. Although the freeze-dried WSPFE exhibited a marginally higher phenolic content and enhanced antioxidant properties, it also involved higher production costs and extended drying times compared to spray drying. Additionally, the spray-dried WSPFE demonstrated lower hygroscopicity and moisture content, making spray drying a more viable option for large-scale and industrial applications of WSPFE.

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