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Preparation of turmeric powder with various extraction and drying methods



Junyoung Park^{1,2}, Subeen Do^{1,2}, Minju Lee^{1,2}, Seungwoo Ha^{1,2} and Kwang-Geun Lee^{1,2*}

Abstract

Background: In this study, turmeric (*Curcuma longa* L.) powder was prepared with various extraction and drying methods. Extractions were performed using three solvents (water, 50% ethanol, and 70% ethanol) and three drying methods, namely, spray drying (SD), freeze drying (FD), and spray-freeze drying (SFD).

Results: Levels of the curcuminoids, curcumin, demethoxycurcumin, and bisdemethoxycurcumin, in turmeric powder preparations were analysed using HPLC, and scanning electron microscopy and laser particle size analysis were used to observe and estimate the surface and size distribution of particles. The total amount of curcuminoids ranged from 0.59–14.35 mg/g turmeric powder, and as the concentration of ethanol increased, the level of curcuminoids, antioxidant activity, and average particle size significantly increased (all p < 0.05). In addition, the abundance of curcuminoids significantly increased in the order of SFD, FD, and SD (p < 0.05), whereas the average particle size significantly increased in the order of FD, SFD, and SD (p < 0.05).

Conclusions: The highest TPC and TFC were detected in SFD turmeric powder preparations, whereas the average particle size significantly increased in the order of FD, SFD, and SD. Based on this study, we also showed that the level of curcuminoids, antioxidant activity, TPC, TFC, and average particle size were most affected by the extraction solvent.

Keywords: Curcuma longa L., Curcuminoids, Extraction, Drying method, Antioxidant activity, Particle

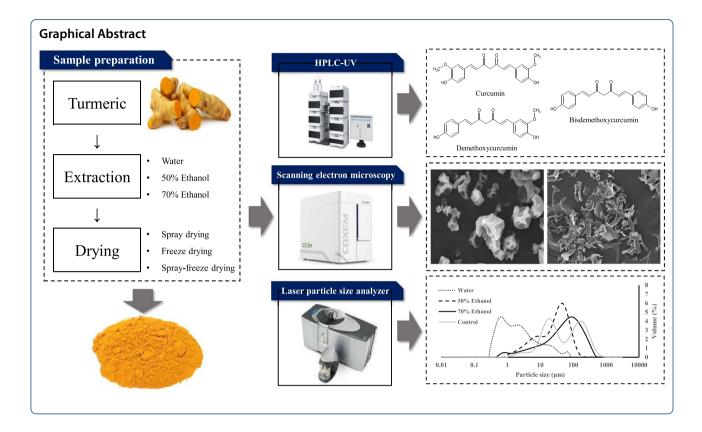
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Introduction

Turmeric (*Curcuma longa* L.), which is a member of the Zingiberaceae family, is a perennial herb that is widely cultivated throughout tropical and subtropical regions of the world, such as India and China [1]. Turmeric powder is used as a spice and is an essential component of curries. It also adds flavour and colour to rice, pasta, meat and vegetable dishes, and salads [2].

Turmeric powder is a well-known spices containing natural antioxidants and especially yellow pigment of turmeric powder is a phenolic pigment, which is an antioxidant that can scavenge superoxide radicals, hydrogen peroxide and nitric oxide [3]. In addition, Turmeric powder has numerous health benefits, including antiprotozoal, anti-inflammatory, anti-venom activities and anti-tumour activity, and is used widely in the cosmetics and pharmaceutical industry [4]. Major bioactive components of turmeric are curcuminoids, such as curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). Curcuminoids are hydrophobic and readily soluble in organic solvents, such as methanol, ethanol, and acetone. Among organic solvents, ethanol was reported to have the highest extraction yield [5].

In food processing, freeze drying (FD) and spray drying (SD) are commonly used. Each of various drying methods has its own strength and weakness, and these methods have their own particular characteristics. Although FD can help maintain product quality, such as nutrition, color, and flavor, it entails high production costs, long times, and high energy consumption. Compared to other drying methods, the particles of FD are larger and more uniform [6]. SD is a technology providing with a dry powder product that rapidly evaporates water from the liquid. It is supplied to the spray chamber and has been used in many industries for decades. Hot air is drawn into the chamber to evaporate water from the atomized particles and convert the liquid to a dry powder. SD is advantageous in that it produces free flowing powders in a controlled particle size range from fast drying. Despite these positive aspects, this method can suffer from significant loss of volatiles and thermal degradation of heatsensitive materials due to high inlet temperature [7]. Whereas spray freeze drying (SFD), which combines SD and FD, is a new method for producing powder formulations. It is profitable to have a fast freezing rate, because small, dried particulates and fine, microporous structures develop [8-12].

In this study, turmeric powder was dried with three drying methods (SD, FD, and SFD) using three solvents (water, 50% ethanol, and 70% ethanol). To investigate the physicochemical properties of these different turmeric powder preparations, levels of curcuminoids, antioxidant activity, total phenolic compounds, total flavonoids compounds, and particle size distribution were analysed.

Materials and methods

Chemical reagents and materials

Commercial grinded turmeric (*Curcuma longa* L.) was provided by Jindo Nonghyup in Korea. HPLC-grade water, methanol, acetic acid, chloroform, and acetonitrile were purchased from J. T. Baker (Philipsburg, NJ, USA). CUR (98% purity), DMC (95% purity), and BDMC (95% purity) were purchased from Supelco Corp. (Bellefonte, PA, USA). Primary secondary amine (PSA) was purchased from Agilent (Santa Clara, CA, USA), whereas Folin–Ciocalteu reagent and the antioxidant activity reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All chemicals and reagents used in the study were analytical grade.

The extraction process

The extraction process was modified from the previous study [13]. Ground turmeric (50 g) was extracted with 500 mL of water, 50% ethanol, or 70% ethanol for 2 h at 100 °C. The extract was centrifuged at $6500 \times g$ and 4 °C for 10 min, and the supernatant was filtered through filter paper (No. 3, 110 mm, Whatman). The filtered solution was concentrated using a rotary evaporator under reduced pressure at 60 °C to remove ethanol.

The drying process

SD, FD, and SFD process were followed by the previous reports [9-12]. SD was performed in a B-290 spray dryer (Buchi, Flawil, Switzerland). The extract was fed into the chamber using a peristaltic pump, controlling the feed flow rate using the pump rotation speed. The air and feed flow rates were 5 bars and 10 mL/min, respectively. The inlet and outlet temperatures were 150 °C and 85 °C, respectively.

For FD, the extract was frozen in an SE-45 deep freezer (Tefcold, Viborg, Denmark) at - 80 °C for more than 24 h and then dried using a FD 8508 freeze dryer (Ilsin Lab Co., Seoul, Korea) at- 70 °C and under 5 mTorr pressure for 7 days.

For SFD, the solution was sprayed with a feed rate of 43 mL/min and immediately frozen in liquid nitrogen. Drying was performed at a pressure of 0.05 mbar. The shelf temperature was kept at -20 °C for 28 h and was then increased to 20 °C for 16 h. Before analysis, dried powders were stored at -80 °C in a deep freezer.

Analysis of curcuminoids

Sample and ethanol were added to a 50 mL teflon centrifuge tube, and ultrasonic extraction was performed for 3 h at 50 °C. After the addition of PSA (0.2 g), the extract was then centrifuged at 14,000 × g and 4 °C for 15 min. The supernatant was filtered through a 0.45 μ m nylon filter.

Curcuminoid qualification was performed using thin layer chromatography (TLC) and HPLC. For TLC, 20 µL of sample was manually spotted on a pre-coated silica gel 60-F254 aluminium plate. The distance between each spot was 1 cm. The plate was dried at room temperature and then developed in a TLC chamber previously saturated with chloroform: methanol (47:3, v/v). Bands were scanned at 365 nm. The conditions of HPLC was optimized based on [14]. HPLC was performed using a 1200 Series Gradient system (Agilent Technologies, Santa Clara, CA, USA) equipped with a UV-VIS detector (UVD). The separation of curcuminoids was performed using octadecyl-silica (C18) $(4.6 \times 250 \text{ mm}, 5 \text{ }\mu\text{m})$. The column temperature was set at 30 °C, and 2% acetic acid (45% acetonitrile) was used as the mobile phase. The flow rate was constant at 1 mL/min, and the injection volume was 20 µL. The detection wavelength was 425 nm.

Analysis of antioxidant activity

The antioxidant activity of turmeric powder preparations was determined using DPPH and ABTS assays.

DPPH assay

The DPPH free radical scavenging activity of extracted samples was determined according to [15]. DPPH reagent (0.1 mmol L⁻¹) was diluted with methanol to obtain an absorbance of 1.0 ± 0.02 at 517 nm using a spectrophotometer. The DPPH solution (2.9 mL) was mixed with 0.1 mL of turmeric powder dissolved in methanol and then incubated for 30 min in the dark. The absorbance at 517 nm was determined using an UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Ascorbic acid was used as a positive control. The percentage of DPPH inhibition was calculated using the following equation:

% DPPH radical scavenging activity = $[(Abs_{blank} - Abs_{sample})/Abs_{blank}] \times 100$ where Abs_{blank} and Abs_{sample} are the absorbance values of methanol and sample, respectively.

ABTS assay

The ABTS cation radical scavenging assay was followed [16]. ABTS reagent (7.4 mmol L⁻¹) and potassium persulfate solution (2.6 mmol L⁻¹) were mixed in equal amounts and reacted in the dark at room temperature for 16 h. Next, ABTS solution (1 mL) was diluted with methanol to obtain an absorbance of 1.0 ± 0.02 at 734 nm using a spectrophotometer. ABTS solution (2850 µL) was mixed with 150 µL of turmeric powder dissolved in methanol and then incubated for 2 h in the dark. The absorbance was measurement at 734 nm, and ascorbic acid was used as a positive control. The percentage of ABTS inhibition was calculated as follows:

% ABTS radical scavenging $activity = [(Abs_{blank} - Abs_{sample})/Abs_{blank}] \times 100$ where Abs_{blank} and Abs_{sample} are the absorbance values of methanol and sample, respectively.

Analysis of total polyphenol content (TPC) and total flavonoid content (TFC)

Sample extraction for analysis of TPC and TFC

The method of extraction was similar to the method of [17]. Turmeric powder was extracted with 10 mL of methanol: water (80:20) and stirred for 12 h. The extract was then re-extracted with 10 mL of the same solvent and filtered through a 0.45 μ m nylon filter.

ТРС

Folin–Ciocalteu reagent was used for TPC according to [18]. The extract (300 μ L) was mixed with 2250 μ L of 10% Folin–Ciocalteu reagent. After 5 min of equilibrium, 6% sodium carbonate solution (2250 μ L) was added. After 90 min of reaction, absorbance was measured at 725 nm using a spectrophotometer. A calibration curve of gallic acid was prepared, and the result was expressed as milligram of gallic acid equivalent per gram of turmeric powder (mg GAE/g turmeric powder).

TFC

The total flavonoid content was determined using the colourimetric method described by [19]. For TFC, the extract (1000 μ L) was mixed with 400 μ L of 5% sodium nitrate. The mixture was allowed to stand for 6 min, followed by the addition of 10% aluminium chloride (400 μ L). After 6 min, 4% sodium hydroxide (4000 μ L) was added. Methanol was used to adjust the volume to 10 mL. Next, the mixture was incubated for 15 min and absorbance was measured at 510 nm using a spectrophotometer. A calibration curve of quercetin was prepared, and the result was expressed as milligram of quercetin equivalent per gram of turmeric powder (mg QCE/g turmeric powder).

Scanning electron microscopy (SEM)

The particle surface was observed by SEM using an EM-30 scanning electron microscope from COXEM (Daejeon, Korea). Each dried powder was mounted on an aluminium stage using carbon tape and then coated with gold for 3 min.

Analysis of particle size distribution

A laser particle size analyser (Mastersizer 3000, Malvern Panalytical, Malvern, UK) was used to measure the

particle size distribution. Turmeric powder (10 mg) was interspersed in 40 mL of water and then analysed.

Statistical analysis

All analyses in this study were performed in triplicate and expressed as mean \pm standard deviation (SD). Comparisons were performed by analysis of variance (ANOVA), followed by Duncan's multiple range test using IBM SPSS Statistics for Windows version 25 (IBM Corp., Armonk, NY, USA). Principal component analysis (PCA) was performed to visualise the distribution of samples according to the various extraction and drying methods using XLSTAT (Addinsoft, New York, NY, USA). A *p* value less than 0.05 was considered statistically significant.

Results and discussion

Analysis of curcuminoids by TLC and HPLC

Additional file 1: Fig. S1 shows the TLC analysis of each sample's result using an UV lamp. The chromatogram of curcuminoids using HPLC-UVD is shown in Additional file 1: Fig. S2. The calibration curve of each curcuminoid (CUR, DMC, and BDMC) showed good linearity (all $R^2 = 1.000$) at a concentration range of $0.1 - 25.00 \ \mu g/$ mL. The content of curcuminoids in our turmeric powder preparations is shown in Table 1. The total amount of curcuminoids ranged from 0.945–14.21 mg/g turmeric powder, and the level of CUR, DMC, and BDMC ranged from 0.611-9.443, 0.182-2.704, and 0.151-2.06 mg/g turmeric powder, respectively. We found that the curcuminoid with the highest content was CUR, whereas the lowest content was BDMC. These findings are similar to those reported in a previous study [6]. Furthermore, the abundance of CUR was approximately 4 to 10 times higher than DMC and BDMC in turmeric powder.

Curcuminoid extraction is greater in ethanol than in water. In this study, the content of curcuminoids increased significantly with higher ethanol concentration in the extraction solvent for each drying method (all p < 0.05). Furthermore, the abundance of curcuminoids significantly increased in the order of SFD, FD, and SD (p < 0.05). In the various previous reports, curcuminoids are efficiently extracted in organic solvents [20], whereas heat treatment of turmeric results in a significant loss [21, 22].

Analysis of antioxidant activity, TPC, and TFC

Turmeric is rich in phenolic compounds, and the antioxidant activity of turmeric has been intensively studied [23–25]. Both polyphenol compounds and flavonoid compounds have a major influence on determining antioxidant activity [26].

The antioxidant activities of nine turmeric powder preparations were measured by DPPH and ABTS assays

Table 1 Concentration of curcuminoids in tur	heric powder according to the	e various extract and drying methods

Sample type	Curcuminoids (mg/g turmeric powder)					
	CUR	DMC	BDMC	Total		
Control	1.233 ± 0.002^{d}	0.302 ± 0.002^{d}	0.221 ± 0.004^{c}	1.756 ± 0.007^{d}		
Spray drying						
Water	0.611 ± 0.002^{a}	0.182 ± 0.001^{a}	0.151 ± 0.002^{a}	0.945 ± 0.003^{a}		
50% ethanol	3.448 ± 0.004^{e}	$0.865 \pm 0.004^{\rm e}$	0.544 ± 0.008^{d}	4.86 ± 0.02^{e}		
Freeze drying						
Water	0.798 ± 0.002^{b}	0.247 ± 0.001^{b}	0.206 ± 0.003^{b}	1.251 ± 0.003^{b}		
50% ethanol	4.075 ± 0.003^{f}	1.089 ± 0.004^{g}	0.736 ± 0.007 ^f	5.90 ± 0.01 ^f		
70% ethanol	8.34 ± 0.02^{h}	2.399 ± 0.006^{h}	1.836 ± 0.009^{h}	12.58 ± 0.03^{h}		
Spray-freeze drying						
Water	$0.892 \pm 0.003^{\circ}$	$0.276 \pm 0.001^{\circ}$	$0.230 \pm 0.003^{\circ}$	$1.397 \pm 0.003^{\circ}$		
50% ethanol	4.227 ± 0.005^{g}	$1.060 \pm 0.005^{\rm f}$	0.67 ± 0.01^{e}	5.95 ± 0.02^{g}		
70% ethanol	9.443 ± 0.009^{i}	2.704 ± 0.006^{i}	2.06 ± 0.02^{h}	14.21 ± 0.02^{i}		

1) All values are represented as mean \pm standard deviation (S.D.) (n = 3)

2) Different superscript letters in the same column denote significant differences (Duncan's range test, p < 0.05)

Table 2 Antioxidant activities in turmeric powder according to the various extract and drying methods

Sample type	DPPH radical scavenging activity	ABTS radical scavenging activity
Control	8 ± 1^{a}	14.8 ± 0.8^{a}
Spray drying		
Water	8.5 ± 0.9^{a}	$27.9\pm0.8^{\rm b}$
50% ethanol	17.7 ± 0.9^{b}	62 ± 2^{c}
Freeze drying		
Water	8 ± 1^{a}	$29.6\pm0.7^{\rm b}$
50% ethanol	17 ± 1^{b}	62 ± 3^{c}
70% ethanol	23 ± 1^{c}	67 ± 3^{d}
Spray-freeze drying		
Water	9 ± 1^{a}	$26.0\pm0.9^{\rm b}$
50% ethanol	16 ± 1^{b}	59 ± 2^{c}
70% ethanol	25 ± 2^{c}	68 ± 4^d
Ascorbic acid	40 ± 2^d	$98 \pm 4^{\text{e}}$

1) All values are represented as mean \pm standard deviation (S.D.) (n = 3)

2) Different superscript letters in the same column denote significant differences (Duncan's range test, p < 0.05)

using ascorbic acid as a positive control (Table 2). We found that the antioxidant activity differed according to the extraction solvent. For each drying method, the antioxidant activity increased as the ethanol concentration increased. This finding was expected, because curcuminoid abundance increases with increasing ethanol concentration. In vitro study reported previously, curcuminoids such as curcumin have strong antioxidant activity [27]. Because SD occurs at high temperature, the content of curcuminoids is decreased. However, as heat **Table 3** Contents of total phenolics, total flavonoids contentin turmeric powder according to the various extract and dryingmethods

Sample type	TPC (mg GAE/g turmeric powder)	TFC (mg QCE/g turmeric powder)
Control	8.7 ± 0.3^{a}	9.5 ± 0.8^{a}
Spray drying		
Water	10.8 ± 0.4^{b}	10.9 ± 0.6^{a}
50% ethanol	30 ± 1^{d}	43 ± 1^{b}
Freeze drying		
Water	$13.4 \pm 0.4^{\circ}$	9.4 ± 0.7^{a}
50% ethanol	32 ± 2^{e}	43 ± 2^{b}
70% ethanol	36.9 ± 0.2^{f}	69 ± 1^{d}
Spray-freeze drying		
Water	$13 \pm 1^{\circ}$	10.4 ± 0.8^{a}
50% ethanol	32 ± 1^{e}	47 ± 2^{c}
70% ethanol	38.6 ± 0.2^{g}	$71.8\pm0.7^{\rm e}$

1) All values are represented as mean \pm standard deviation (S.D.) (n = 3)

2) Different superscript letters in the same column denote significant differences (Duncan's range test, p < 0.05)

treatment increases free polyphenols, which affect the antioxidant activity, we found no significant difference in antioxidant activity among the three drying methods [28].

For TPC and TFC analysis, calibration curves of gallic acid and quercetin showed linearity ($R^2 = 0.992$ and 0.991, respectively) at a concentration range of 0.1 – 100.0 µg/mL. Based on our analysis, we found that TPC and TFC in turmeric preparations differed according to the extraction solvent (Table 3). For each drying condition, TPC and TFC increased with increasing concentrations of

sample extracted using 70% ethanol. Water is not an efficient solvent, because polyphenol compounds are more soluble in less polar solvents than water [29]. Although curcuminoids are not classified as flavonoids, they react similarly as flavonoid compounds to the aluminium chloride (AlCl₃) used in the TFC method [30]. The principle in determining TFC is that AlCl₃ forms acid-stable complexes with the keto and hydroxyl groups of flavonoids (Additional file 1: Fig. S3). Curcuminoids are natural diarylheptanoids, which consist of two aromatic o-methoxy phenolic groups linked by a seven-carbon chain [30]. They exist in either the enol or keto form; however, the enol configuration is the major component in solution [31]. AlCl₃ binds to curcuminoids through the β -diketone group (Additional file 1: Fig. S4) [32].

Evaluation of morphology and particle size distribution

As shown in Fig. 1, we evaluated the particle size distribution of turmeric powder preparations and found that all turmeric extracts subjected to a drying process have a more uniform particle size than the control. Furthermore, the average particle size of turmeric extracted using water was smaller than those using ethanol extraction. In addition, for each extraction solvent, we found that turmeric preparations by FD resulted in the largest average particle size, whereas SD resulted in the smallest (Table 4) [33, 34].

Next, the physical structure of turmeric powder particles was observed using SEM. As shown in Fig. 2, we found that the particle structure of turmeric powder preparations was completely different depending on the drying method. Specifically, the powder from SD samples demonstrated a distorted spherical-like particle shape (Fig. 2b, c), whereas those from FD samples formed a flaky powder (Fig. 2d–f) [35]. In contrast, we found that SFD samples have a spherical particle shape (Fig. 2g–i).

These findings are mainly caused by the atomisation during SD and SFD that results in smaller droplets, and

Table 4	Particle	sizes	(diameter,	μm)	in	turmeric	powder
accordin	g to the v	various	extract and	dryin	g m	ethod	

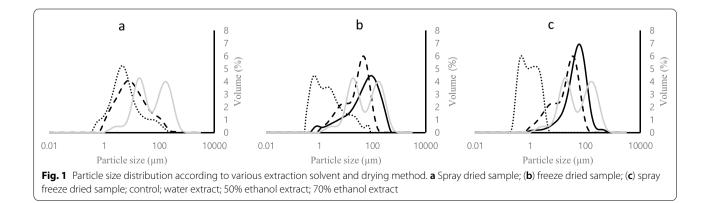
Sample type	Mean (µm)	Median (µm)	
Control	102.72	51.36	
Spray drying			
Water	1.21	0.61	
50% ethanol	23.36	11.68	
Freeze drying			
Water	13.81	6.91	
50% ethanol	36.53	18.27	
70% ethanol	85.24	42.62	
Spray-freeze drying			
Water	5.86	2.93	
50% ethanol	28.30	14.15	
70% ethanol	64.85	32.42	

these atomised droplets are often spherical in shape. In the previous reports, the low temperature involved in FD and a lack of strength to break the frozen drops or alterations to the surface during drying may be reasons for the larger particles and flake-like structures [33, 36].

PCA

In general, the applicability of simple statistical descriptors, such as mean, variance, and covariance, decrease with increasing number of variables. As the size and complexity of data increase, such methods fail to accurately explain trends in data. PCA is a well-suited technique for describing large data sets, using a covariance matrix system to do what univariate methods cannot [37].

Quantitative data obtained from curcuminoid abundance, antioxidant activity, TPC, TFC, and particle size were analysed using PCA. Two principal components explained 82.17% and 15.86% of data variance (F1 and F2, respectively). We identified three separate groups according to extraction solvent (Additional file 1: Fig. S5). As a result of measuring curcuminoids, antioxidant activity,



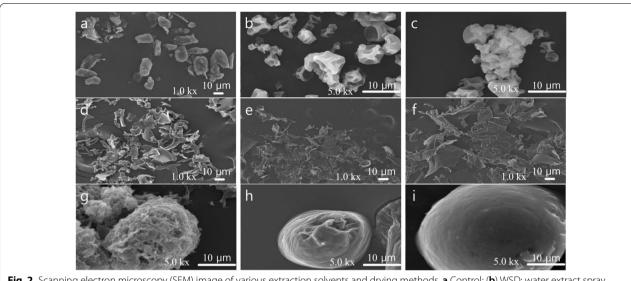


Fig. 2 Scanning electron microscopy (SEM) image of various extraction solvents and drying methods. **a** Control; (**b**) WSD: water extract spray drying; (**c**) 50ESD: 50% ethanol extract spray drying; (**d**) WFD: water extract freeze drying; (**e**) 50EFD: 50% ethanol extract freeze drying; (**f**) 70EFD: 70% ethanol extract freeze drying; (**g**) WSFD: water extract spray freeze drying; (**h**) 50ESFD: 50% ethanol extract spray freeze drying; (**i**) 70ESFD: 70% ethanol extract spray freeze drying; (**i**) 70ESFD:

TPC, and TFC, we found that turmeric powder extracted from 70% ethanol was the closest.

Conclusions

In this study, we investigated the characteristics of turmeric powder prepared with three solvents (water, 50% ethanol, and 70% ethanol) and drying methods (SD, FD, and SFD). We determined the level of curcuminoids, antioxidant activity, TPC, TFC, and particle size in nine different samples, including control, and discovered, as the concentration of ethanol increased, the level of curcuminoids, antioxidant activity, TPC, TFC, and average particle size significantly increased (all p < 0.05). In addition, SFD turmeric powder had higher levels of curcuminoids than SD and FD turmeric powder. However, it was confirmed that the antioxidant activity was not affected by drying method. Furthermore, the highest TPC and TFC were detected in SFD turmeric powder preparations, whereas the average particle size significantly increased in the order of FD, SFD, and SD. Based on PCA, we also showed that the level of curcuminoids, antioxidant activity, TPC, TFC, and average particle size were most affected by the extraction solvent.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-022-00307-1.

Additional file 1: Figure S1 TLC of turmeric powder according to the various extract and drying methods. STD: Standard; WSD: Water extract Spray Drying; 50ESD: 50% Ethanol extract Spray Drying; WFD: Water extract Freeze Drying; 50EFD: 50% Ethanol extract Freeze Drying; 70EFD: 70% Ethanol extract Freeze Drying; WSFD: Water extract Spray Freeze Drying; 50ESFD: 50% Ethanol extract Spray Freeze Drying; 70ESFD: 70% Ethanol extract Spray Freeze Drying. Figure S2 HPLC–UV chromatogram of curcuminoids (Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin). Figure S3 Formation of flavonoid complex with AlCl₃. Figure S4 Formation of curcumin complex with AlCl₃. Figure S5 PCA of curcuminoids, antioxidant activity, TPC, TFC, and particle size. WSD: water extract spray drying; 50ESD: 50% ethanol extract spray drying; WFD: water extract freeze drying; 50EFD: 50% ethanol extract freeze drying; 70EFD: 70% ethanol extract freeze drving: WSFD: water extract sprav-freeze drving: 50ESFD: 50% ethanol extract spray-freeze drying; 70ESFD: 70% ethanol extract spray-freeze drying.

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Not applicable.

Author contributions

[38] JP: formal analysis, investigation, and methodology; SD: formal analysis; ML: validation; SH: methodology; KGL: supervision, validation, investigation, and project administration. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

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