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Physicochemical, antioxidant, and anti-tyrosinase property of standardized phenolics powder from mango seed kernel

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ABSTRACT

Mango (*Mangifera indica* L.) is one of the major crops produced in the Philippines. In recent years, the mango industry is facing challenges with issues like infestations, seasonality, and competition, causing a decline in the global market share. To help alleviate the industry, mango by-products, such as seeds and peels, are being actively studied for their polyphenol content, which has potential benefits in pharmaceutical and cosmeceutical applications. In this study, we evaluated the physicochemical and bioactive properties of spray-dried phenolics from mango seed kernel (SPMK) across varying extraction volumes (1800 mL, 9000 mL, 14400 mL) using an optimized extraction and spray-drying process. Findings revealed that larger extraction volumes resulted in lower moisture content and higher water solubility index (WSI), indicating improved powder stability and solubility, with constant bulk density and tapped density. The total phenolic content (TPC) was maintained at 138.04 to 141.97 mg GAE/g across extraction volumes, with strong antioxidant activity and significant tyrosinase inhibition (IC_{50}: 6.62 to 6.91 µg GAE), demonstrating the potential of SPMK for high-value applications.

INTRODUCTION

Globally, the Philippines is positioned as one of the top producers and exporters of mango with approximately 2.6% global market share (Centino et al. 2020). Despite this, the Philippine mango industry has been experiencing a gradual decline in its performance that is primarily attributed to infestations, production seasonality, and international market competition (DA-BAR 2022). These challenges have forced mango farmers and processors to shift their focus to alternative

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crops. This situation has prompted researchers to explore innovative solutions to revitalize the industry, one of which involves valorization of underutilized by-products of mango processing.

The processing and consumption of mango yields by-products in the form of seeds and peels, constituting 35-60% of the fruit's total weight (García-Mahecha et al. 2023). These by-products are often underutilized, typically disposed of by unplanned landfilling or incineration which poses deleterious environmental effects. Nonetheless, previous efforts have highlighted the potential of mango by-products as a valuable source of polyphenolic compounds (Tacias-Pascacio et al. 2022). These bioactive compounds are now being actively studied, not only for their applications in the prevention of chronic diseases, but also for their promising potential in promoting skin health. Polyphenols or phenolics are known for their antioxidant properties that inhibit the upregulation of free radicals, which are involved in the degradation of collagen and elastin, the proteins responsible for skin's firmness and elasticity (Imokawa and Ishida 2015; Mohiuddin 2019; Tobin 2017). Additionally, the structural features of phenolics account for their high affinity toward amino acids and their inherent metal-chelating property, allowing them to modulate the activity of specific dermal enzymes and play a significant role in mitigating premature skin aging and hyperpigmentation issues (Mechqoq et al. 2022; Yu et al. 2019).

Despite their potential, the extraction of polyphenols poses significant challenges due to their structural complexity and instability under various processing conditions. Phenolics are particularly prone to degradation due to exposure to heat, light, oxygen, and variations in pH which can lead to loss of desirable physicochemical properties and bioactivity (Antony and Farid 2022). This problem is further magnified when production of polyphenols is scaled up. In larger production volumes, maintaining uniformity and consistency in extraction arises as a major concern. Likewise, the variability of raw materials, especially non-conventional sources such as mango by-products, can lead to inconsistencies in phenolic content, affecting the standardization of chemical composition. To address these issues, process optimization is usually performed to identify parameters that can affect the recovery of phenolic compounds. In our previous study, we have reported that solids loading, solvent concentration, and extraction time as the main factors affecting the total phenolic content (Sapin et al. 2020). Optimizing these parameters can bridge the transition from lab-scale to industrial-scale production of phenolics.

At present, a phenolic-based cosmetic ingredient is being developed to harness its antioxidant properties and potential skin-brightening effects. In this study, the bioactivities and physicochemical properties of the standardized phenolic powder (spray-dried phenolics from mango seed kernel, SPMK) were assessed based on increasing production volumes. The total phenolic content (TPC), antioxidant capacity, as well as anti-tyrosinase activity, were evaluated to rationalize its cosmeceutical applications.

MATERIALS AND METHODS

Production of spray-dried phenolics from mango seed kernels (SPMK)

Mango seeds of the *Carabao* variety were obtained from several mango plant processors in Luzon (National Capital Region, Cavite and Bulacan Regions), Philippines. The mango seed husks were manually removed to obtain the kernels, which were subsequently dried at 60ºC for 24 hours in a custom-fabricated convective air-drying oven. The dried samples were ground using a grinder (Retsch GM 100) and passed through a 30-mesh sieve (Tyler) to obtain homogenized particle sizes.

Phenolic constituents of the mango seed kernel powder (MSKP) were extracted using optimized solvent-assisted production parameters. The MSKP at 15% (w/v) solids loading was suspended in a binary solvent consisting of ethanol and water (60 % v/v concentration) and mixed for 1.5 hours using a fabricated mechanical shaker. Suspended solids were removed via centrifugation at 9,000 rpm and 25ºC for 20 minutes (Hanil Combi 514 R) and the extracts (supernatants) were concentrated and removed of the solvent by rotary evaporation at 50ºC and 150 rpm (Heidolph Hei-Vap). The concentrated extract was diluted and mixed with maltodextrin at 15% (w/v) loading for 3 hours. The encapsulated extract was then subjected to low-temperature spray dryer set to 50°C (YK-100, True Ten Industrial Co. Ltd.) to produce the phenolic extract in powdered form. The production run initially utilized an extraction volume of 1800 mL which was subsequently upscaled to 9000 mL, representing a 5-fold increase and then further increased to 14400 mL, representing an 8-fold increase. With the increase of extraction volume, the extraction conditions—solids loading at 15% w/v, the ethanol concentration (60% w/v) and extraction time of 1.5 hours, were maintained. However, for the spray drying, the higher production volumes (9000 and 14400 mL) used a high-temperature spray dryer set to 150˚C (LPG5 High Speed Centrifugal Spray Dryer, MachineLab Technology Inc.), which is the standard spray drying equipment in commercial production settings. The SPMK were kept in tightly sealed high-density polyethylene (HDPE) bottles at room temperature until use.

Physicochemical Properties

Moisture. The moisture content (%MC) was determined by drying the SPMK (0.5 grams) in an oven-drier (Carbolite AX60) at 105ºC until constant weight was obtained (±0.0010 g). The loss in weight was taken as the weight of water in the sample (AOAC 2019).

$$Moisture\ Content\ (\%) = \frac{loss\ in\ weight\ (g)}{initial\ weight\ of\ the\ sample\ (g)} x100 \quad (1)$$

Water solubility index. The water solubility index (WSI) of the SPMK was assessed based on the method of Anderson (1982) with some modifications. In 50-mL centrifuge tubes, 1 gram of samples was mixed with 20 mL distilled water. The resulting mixtures were vortexed for 1-2 minutes to break up clumps, then shaken for 30 minutes at room temperature. The undissolved solids were separated by centrifugation at 9000 rpm and 25ºC for 20 minutes (Hanil Combi 514 R). The supernatants were carefully decanted into pre-weighed aluminum pans and were dried in an oven at 105ºC until constant weight was obtained (±0.0010 g). The WSI was calculated using the formula below:

$$WSI\ (\%) = \frac{Weight\ of\ dried\ solids\ recovered\ from\ supernant\ (in\ g)}{Weight\ of\ initial\ sample\ (in\ g)} x\ 100 \quad (2)$$

Bulk and tapped density. The bulk (ρ_{bulk}) and tapped (ρ_{tap}) densities of the powders were determined based on the method of Shishir et al. (2014). Briefly, 2 grams of the SPMK were transferred into a 10-mL graduated cylinder. The volume occupied by the powder was recorded and its ratio to its mass is reported as the ρ_{bulk}. On the hand, tapped density was measured by tapping the graduated cylinder containing the powder until the volume of the powder remained constant. The tapping was done manually by dropping the graduated cylinder from a height of 15 cm until constant volume level was observed. The mass of the powder divided by the volume after tapping was reported as the $\rho_{tap.}$

Color. The variations in color of the spray-dried phenolics were assessed using a Konica Minolta CM-5 colorimeter. SPMK samples were evenly spread in a petri dish and measurements were taken by placing the dish on the device's top-port. The instrument outputs the results in terms of CIE L*a*b* color space. Using the a* and b* scales, chroma (C*) and hue angle (h°) were calculated based on the following equation:

$$Chroma = (a^{*2} + b^{*2})^{\frac{1}{2}} \quad (3)$$

$$Hue = \left(\frac{b^*}{a^*}\right) \quad (4)$$

Total phenolic content

The determination of total phenolic content (TPC) was conducted using a modified Folin-Ciocalteu method, described elsewhere (Núñez Sellés et al. 2002). The SPMK (300 mg) was first dissolved in ten (10) mL distilled water, mixed for one (1) hour at 480 rpm (IKA RO-5 Power) and centrifuged for 20 minutes at 12,500 rpm (Hanil Combi 514 R) to obtain the spray-dried phenolic extract. In a test tube, 0.5 mL of the diluted extract was mixed with 0.5 mL 1 N Folin-Ciocalteu's phenol reagent and 0.5 mL of 10% Na_2CO_3. The resulting solution was vortexed and allowed to stand at room temperature for 5 minutes. Then 5 mL distilled water was added to dilute the solution .The absorbance was measured in triplicates at a wavelength of 720 nm using an Ultraviolet-Visible spectrophotometer (Shimadzu UV-1601). The TPC was calculated by interpolating the absorbance reading in a gallic acid standard curve and was expressed in terms of gallic acid equivalents (GAE). The standard curve of gallic acid was prepared using gallic acid solutions at concentrations, 20 µg/mL to 100 µg /mL, which generated the standard curve formula y = 0.00678x -0.07275 (R^2 = 0.9991).

Antioxidant Properties

The antioxidant activity of the spray-dried phenolics was determined based on their capacity to scavenge free radicals. The DPPH (2,2-diphenyl 1-picrylhydrazyland) radical scavenging and ABTS (2,2′-azino-di-(3-etylobenzotizoline-6-sulfonate) radical scavenging assays were employed in this regard. Additionally, antioxidant activity was also investigated through the ability of phenolics to induce reduction of active metals that catalyze the formation of free radicals (Huang et al. 2005). This property of antioxidants is usually determined via copper reducing antioxidant capacity (CUPRAC) assay. In this study, DPPH, ABTS, and CUPRAC assays were performed based on the procedure described by Sapin et al. (2021). For all the antioxidant assays, the activity was assessed through the half-maximal effective concentration (EC_{50}). The EC_{50} was determined by interpolation of the % inhibitions (DPPH and ABTS) and absorbance readings (CUPRAC) to obtain the concentration that exhibits 50% inhibition (for DPPH and ABTS) and an absorbance reading of 0.50 (CUPRAC). The intermediate values of inhibitions and absorbance readings were not included in this paper.

DPPH radical scavenging assay. Briefly, in separate test tubes, an aliquot of 100 µL of SPMK extracts at various concentrations (5-10 µg GAE) or ascorbic acid (20-25 µg) was added to five (5) mL of 0.1 mM DPPH methanol solution and left to stand for 20 minutes. Each solution was then vortexed; the absorbance was read at 517 nm with distilled water as the blank. The control tube was comprised of the DPPH solution with distilled water (100 µL). The % DPPH inhibition was calculated as the ratio between the difference of the absorbance of the control and the sample with the absorbance of the control.

ABTS radical scavenging assay. In separate test tubes, an aliquot of 40 uL of SPMK extracts at various concentrations (3-4.5 µg GAE) or ascorbic acid (3-4.5 µg GAE) was added to three (3) mL of the $ABTS^{\bullet+}$ working solution with an initial absorbance of 0.72 ± 0.05 and left to stand for 5 minutes. Each solution was then vortexed, and absorbance was determined at 734 nm with distilled water as the blank. The control tube was comprised of the ABTS solution with 90% methanol (100 µL). The %ABTS inhibition was calculated as the ratio between the difference of the absorbance of the control and the sample with the absorbance of the control.

CUPRAC Assay. In separate test tubes, an aliquot of 0.5 mL of SMPK extracts at various concentrations (5-10 µg GAE) or ascorbic acid (20-25 µg) was added to a solution of 1 mL 0.01M $CuCl_2$, 1.0 mL of 1.0 M $NH_4CH_3CO_2$, and 1.0 mL of 7.5 mM neocuproine, stirred and left to stand for 20 minutes. Then 0.6 mL of distilled water was added to each tube and the solution was vortexed; the absorbance was read at 450 nm with reagent blank comprised of the reagents and water (0.5 mL).

Tyrosine inhibitory activity

The potential of SPMK as skin lightening agents was evaluated by assessing its inhibitory activity against tyrosinase. The study utilized the methodology of Hapsari et al. (2012), with modifications employed by (Sapin, Alaon, et al. 2021). The experiments were performed using 96-well microplates, and the absorbances were read at 490 nm using a microplate reader (BIOBASE ELISA). In each well, 40 µL of 5mM DOPA (3,4-dihydroxy- L-phenylalanine, Sigma D-9628), 40 µL 0.1M potassium phosphate buffer pH 6.5, an aliquot of 40 µL SPMK extracts at various concentrations (4-14 µg GAE)/ kojic acid (1.6-5.6 µg)/ ascorbic acid (1.6-5.6 µg) or buffer for control, and 40 µL mushroom tyrosinase (250 units/mL, Sigma T- 3824) were added in sequence and gently shaken. The solution was left to stand for 15 minutes, and the absorbance was determined. The % inhibition was also calculated as the ratio between the difference of the absorbance of the control and the sample with the absorbance of the control. The relative performance of the phenolic powders was determined by calculating the half-maximal inhibitory concentration (IC_{50}) for each assay and comparing these values to those of kojic acid and ascorbic acid.

Statistical analysis

Prior to analysis, data from each assay were subjected to the Shapiro-Wilk test to check the normality of data distribution and Levene's test to assess the equality of variances among treatment groups. If both assumptions were satisfied, a classical one-way ANOVA followed by Tukey's HSD post hoc test was performed to determine the significance between treatments. In cases where the assumption of unequal variances was violated, Welch's ANOVA followed by the Games-Howell post hoc test was performed. A 5% significance level was adopted for all tests. Measurements were conducted in triplicates, with outliers removed using Grubb's Test to increase the reliability of the data. For each parameter, equal sample sizes were maintained across treatments.

RESULT AND DISCUSSION

Physicochemical Properties

Moisture content. Moisture content (MC) is an important factor that determines the stability and quality of high-value products, specifically powders intended for cosmeceutical and pharmaceutical applications. Table 1 presents the moisture content of the spray-dried phenolics from mango kernel (SPMK) were 2.420% to 5.705%. The ANOVA results showed that the

moisture contents differed significantly ($p < 0.05$). Consequently, applying post-hoc test, we found that SPMK produced at 9000 mL and 14400 mL volumes both resulted in moisture contents with no statistical difference, but both showed a difference with the moisture content of the SPMK produced at 1800 mL ($p < 0.05$). The two higher production volumes resulted in moisture content below 5%, while the highest MC was recorded for SPMK produced at 1800 mL. Aside from the difference in the production volume, the variation might be due to the difference of the spray-drier used.. Due to the limitations of the instrument used for the 1800 mL scale the temperature used was at 50°C whereas for larger scale was at 150°C. The parameters that will be used for further scale-up will be those of the larger scale. The other researchers who have also documented the moisture content of spray dried seed kernel extract, reported values between 3.56% to 5.01% (Siacor et al. 2020) and 2.74 to 5.83 % (Lim et al. 2019). Although in their studies, the extraction volumes they used were not specified.

Table 1: Physicochemical properties of spray-dried phenolics extracted from mango kernels.

Physicochemical property	SPMK at varying production volumes		
	1800 mL	9000 mL	14400 mL
Moisture content*, %	5.705 ± 0.000^{a}	2.470 ± 0.001^{b}	2.420 ± 0.001^{b}
Water solubility index*, %	91.670 ± 0.071^{b}	94.105 ± 0.573^{ab}	96.555 ± 0.092^{a}
Bulk density**, g/mL**	0.296 ± 0.009^{a}	0.335 ± 0.003^{a}	0.317 ± 0.004^{a}
Tapped density**, g/mL**	0.460 ± 0.007^{b}	0.548 ± 0.011^{a}	0.520 ± 0.010^{a}

Presented as mean ± SD; different superscript in the same row indicates statistical significance ($p < 0.05$); analyzed using Welch's ANOVA with Games-Howell test; *n = 2, **n=3

In relation to stability and quality, low moisture content is generally preferred as it correlates with water activity (a_w), which influences the susceptibility of products to microbial growth, and chemical and enzymatic reactions. It has been reported that spray-dried fruits and vegetables with moisture content of less than 5% are free from microbial activity and have longer shelf life (Tontul and Topuz 2017). This aspect can be explored in future studies to determine whether the low moisture content of SPMK produced at 9000 mL and 14400 mL extraction volumes result in greater stability compared to SPMK produced at 1800 mL.

In another aspect of quality, moisture can also affect other physical properties of powders, particularly their flowability. The presence of moisture can result in agglomeration or clumping of small particles (Sandler et al. 2010). This can adversely affect the overall handling, processing, and performance of the powder product.

Water solubility index. The water solubility index (WSI) of SPMK corresponds to their reconstitution behavior (Mahdavi et al. 2016). As seen in Table 1, the solubility of SPMK samples in water is relatively high ranging from 91.670 to 96.555 %, indicating the instant reconstitution of the powders at room temperature. This can be attributed to the use of maltodextrin, a polysaccharide-based carrier that has good water solubility and low viscosity in solutions (Mahdavi et al. 2016). Carriers such as maltodextrin and gum arabic are comprised of simple sugars that have a high degree of hydroxylation, allowing them to form hydrogen bonds with polar solvents such as water. Comparison of the means through one-way ANOVA indicates that the increasing trend in WSI was positively affected by the production volume ($p < 0.05$).

In addition, studies found that the water solubility index is affected by the particle properties. The WSI was found to be correlated to the heterogeneity and polydispersity of particle size, shape, and surface structures (De Meneses Costa Ferreira et al. 2022). For example, smaller particles have a larger surface area which enhances their rate of reconstitution, suggesting greater WSI (Peng et al. 2004). Other factors like moisture content may lead to an increase in particle size, reducing the solubility of powders in water (Bastías-Montes et al. 2019). Since these were not investigated in the present study, further research is recommended to elucidate the relationships of these variables for the produced SPMK.

Bulk and tapped density. The values of the bulk density and tapped density are listed in Table 1. The bulk densities of the spray-dried phenolics ranged from 0.296 to 0.333 g/mL. Welch's ANOVA indicated that the mean bulk densities of the powders were not statistically significant, demonstrating that an increase in production volume did not significantly affect the interparticle interactions leading to variations in bulking properties. On the other hand, the tapped densities ranged from 0.460 to 0.548 g/mL. Unlike bulk density, the tapped density of the powders was substantially affected by the increase in production volume ($p < 0.05$). Results of the non-parametric post hoc analysis showed that the differences in densities of the powders produced at 9000 mL and 14400 mL were not statistically significant, and both had relatively higher tapped densities than the SPMK produced at 1800 mL.

Color. Color plays a significant role in determining product stability and overall characteristics, particularly in industries such as beauty and personal care, where visual acceptance is highly regarded. It is essential to ensure that ingredients do not interfere to maintain the desired color profile in cosmetic formulations. In this study, we measured the color differences of spray-dried phenolics extracted from mango kernel using a quantifiable color space. Specifically, this study focused on analyzing the CIELab color space which is based on Cartesian coordinates. L* represents the lightness ranging from black (L = 0) to white (L = 100), a* and b* are chromaticity indices, indicating red-green axis (+100 to -100) and yellow-blue axis (+100 to -100), respectively. The calculated C* and h scales are part of the CIELCh color system where C* represents chroma or color intensity (deviation from neutral gray), and h represents hue angle, indicating the direct color of the powder.

As seen in Table 2, the values of C* are similar to the b* values, indicating correlation between these parameters. The positive values of b* indicate that the colors of the powder were heading toward yellow, and its intensity increases as the production volume increases, which was confirmed by statistical analysis ($p < 0.05$). The a* scale, meanwhile, has a low contribution to the overall color of the powders due to its near-zero values. This demonstrates that visually the powders appear more yellow to the human eye. This was further confirmed by the hue angles ($86.57^{o} – 91.15^{o}$) which fall within the yellow group (Setha et al., 2023). The evaluation of the L* scale also showed that the powders were generally lighter in color, which can be attributed to the white color of the wall material used to encapsulate the phenolic extract (Parvez et al. 2022). Statistical analysis showed that all the parameters were significantly affected by the increase in extraction volume. Moreover, a post hoc test demonstrated that color parameters of SPMK produced at 9000 mL and 14400 mL showed no statistical significance, and hence should appear

similar.

Table 2: Color profile of spray-dried mango seed kernel phenolics at varying production volume.

Production Volume	L*	a*	b*	C*	h°
1800 mL	89.65[a]	-0.30[c]	14.92[b]	14.92[b]	91.15[b]
9000 mL	80.96[b]	0.87[b]	23.30[a]	23.32[a]	87.86[a]
14400 mL	80.71[b]	1.45[a]	24.15[a]	24.19[a]	86.57[a]

Different superscript in the same column indicates statistical significance ($p < 0.05$); analyzed using Welch's ANOVA with Games-Howell test; n = 3

Total phenolic content

Mango seed kernels are abundant sources of polyphenolic compounds, including phenolic acids (e.g., gallic acid, caffeic acid, ferulic acid, cinnamic acid), flavonoids (e.g., quercetin, catechin, rutin, kaempferol), mangiferin, tannins, and their derivatives. These bioactive compounds have been extensively investigated for their potential health benefits (Choudhary et al. 2022; Masibo and He 2008). In the present study, we employed the Folin-Ciocalteu colorimetric method to quantify the total phenolic content (TPC) of the SPMK.

Our results demonstrate that the TPC of the powders varied from 138.036 mg GAE/g to 141.971 mg GAE/g as shown in Table 3. This range is notably higher than the TPC reported for spray-dried phenolics from mango seed kernels in previous studies. Specifically, Lim et al. (2019) determined a TPC range of 77-127 mg GAE/g, while Siacor et al. (2020) reported a range of 50.62 - 96.14 mg GAE/g.

Table 3: Total phenolic content of the spray-dried phenolics.

Property	SPMK at varying production volumes		
	1800 mL	9000 mL	14400 mL
Total Phenolic Content (mg GAE/g)	138.04 ± 0.31[b]	139.59 ± 0.83[ab]	141.97 ± 1.55[a]

Different superscript in the same row indicates statistical significance ($p < 0.05$); analyzed using Welch's ANOVA with Tukey test; n = 3

It was also determined that the slight increase in TPC with an increase in production volume was statistically significant. The same trend was observed by Gawałek (2021), noting high retention rates of phenolics despite the increase in production volume. Further analysis revealed that a 5-fold increase in production volume from 1800 mL to 9000 mL did not significantly affect the phenolic content ($p > 0.05$ Meanwhile, statistical comparison of the TPC means of SPMK produced at larger volumes (14,400 mL and 9,000 mL) indicates that they are not significantly different. These findings suggest that scaling up the production does not cause significant loss in phenolic content. This can be further studied by producing SPMK at pilot-scale level.

Antioxidant properties

Free radical scavenging capability. Polyphenols can neutralize free radicals (X•) via single electron transfer (SET) or hydrogen atom transfer (HAT), thereby interrupting chain reactions leading to oxidative damage. The reduction reaction produces phenoxyl radicals (ArO• and Ar•$^{+}$) which are relatively stable due to resonance stabilization induced by the aromatic rings and therefore less reactive than X• (Bhuyan and Handique 2022). This property of polyphenols can be probed spectrophotochemically through decolorization methods such as DPPH and ABTS assays.

In our study, the DPPH assay results indicated that SPMK exhibited radical scavenging activity, with EC_{50} ranging from 10.730 µg GAE to 12.700 µg GAE. Through one-way ANOVA, we found that the antioxidant activity of the SPMK was significantly influenced by an increase in the extraction volume ($p < 0.05$). In the subsequent post hoc test, (see Table 4), it was demonstrated that increasing the production from 1800 mL to 9000 mL (5-fold increase) did not significantly affect the antioxidant activity of SPMK. However, further increasing the production volume by 8 folds has significantly lowered the potency (EC_{50} = 12.70 µg GAE; $p < 0.05$). On the other hand, SPMK also showed substantial scavenging activity against ABTS•$^{+}$ that ranged from 2.873 µg GAE to 3.303 µg GAE (Table 4). Assessing the effect of production upscaling showed that a 5-fold or 8-fold increase did not significantly influence the antioxidant capacity of SPMK.

Table 4: Half-maximal effective concentration (EC50) of the antioxidant properties of the spray-dried phenolics and ascorbic acid.

Antioxidant Assay	EC_{50} (µg*)			
	SPMK at varying production volumes			Ascorbic acid
	1800 mL	9000 mL	14400 mL	
DPPH Inhibition Assay**	10.73 ± 0.24[c]	10.75 ± 0.05[c]	12.70 ± 0.17[b]	23.62 ± 0.26[a]
ABTS Inhibition Assay***	2.95 ± 0.01[bc]	3.30 ± 0.12[b]	2.87 ± 0.10[c]	4.34 ± 0.01[a]
CUPRAC Assay***	8.29 ± 0.04[b]	7.72 ± 0.14[c]	7.47 ± 0.08[c]	25.70 ± 0.32[a]

Different superscript in the same row indicates statistical significance ($p < 0.05$); analyzed using one-way ANOVA with post-hoc ** Tukey test and *** Games-Howell test; n = 3; *spray-dried phenolics are expressed in µg GAE—SPMK concentrations µg GAE/g: 138.04 (1800); 139.59 (9000); 141.97 (14400).

Reducing capacity. The reducing capacity (also known as reducing power) is used as an index of the electron-donating ability of antioxidants. This is an important parameter since antioxidants participate in redox reactions that result in the reduction of transition metals (i.e., Cu and Fe) and free radicals that can catalyze the production of reactive oxygen species as previously discussed (Munteanu and Apetrei 2021). The copper-reducing antioxidant capacity (CUPRAC) is the widely used test

for this parameter. The CUPRAC assay is based on the reduction of Cu(II)-neocuproine to Cu(I)-neocuproine, which has λ_{max} of 450 nm. As shown in Table 4, the half-maximal effective concentration (EC_{50}) values of the SPMK in reducing copper ranged from 7.720 µg GAE to 8.290 µg GAE. The reducing capacity of SPMK was significantly affected by the increase in extraction volume ($p < 0.05$). Interestingly, higher production volumes have lower EC_{50} than the initial 1800 mL sample.

In general, the results of the antioxidant analyses indicated that a moderate increase in the extraction volumes slightly affects the radical scavenging property as well as the reducing power of the produced SPMK. In addition, it is also notable that SPMK exhibited lower half-maximal effective concentration (EC_{50}) values compared to ascorbic acid in all antioxidant assays indicating better radical scavenging activity and reducing capacity. This suggests that SPMK can be a good antioxidant ingredient in the cosmeceutical and pharmaceutical industry.

Meanwhile, the antioxidant properties exhibited by SPMK extracts can be accurately attributed to the presence of polyphenols in the SPMK. Polyphenols are known as free radical scavengers and chelators of pro-oxidative metals. Structurally, polyphenols are highly conjugated systems with high degree of hydroxylation that allows them to act as hydrogen and electron donors that effectively neutralize free radicals, reducing the rate of oxidation of important biomolecules (De Mello Andrade and Fasolo 2014; Tsao 2010). In addition, it is also likely that compounds extracted from mango kernels have synergistic action for antioxidant activity. For instance, Berardini et al. (2005) observed that the polyphenol-rich crude extract from mango has better antioxidative capability when compared to pure phenolic standards. Similar findings are also present in our previous studies of the other non-food parts of mangoes (Sapin et al. 2021a; 2021b).

Tyrosinase inhibition has gained significant attention in the field of cosmetic and medical research, as it can induce skin lightening effects and address hyperpigmentation issues. Essentially, tyrosinase is the enzyme that catalyzes the first two steps in melanin biosynthesis. It promotes the hydroxylation of L-tyrosine to L-DOPA (monophenolase activity) and the oxidation of L-DOPA that produces dopaquinone (diphenolase activity) which undergoes cyclization forming dopachrome (Likhitwitayawuid 2008). Many in vitro assays monitoring tyrosinase inhibition are devised based on the enzyme's diphenolase activity since the dopachrome produced is optically sensitive around 475 nm (Fan et al. 2021).

Through a spectrophotometric assay, we determined that spray-dried phenolics extracted from mango kernel (SPMK) exhibit concentration-dependent inhibitory activity against tyrosinase (see Figure 1). In addition, the half-maximal inhibitory concentration values (IC_{50}) of SPMK extracts were found to range from 6.62 µg GAE to 6.91 µg GAE (Table 5). The inhibitory property of SPMK on tyrosinase can be attributed to its polyphenol content. Polyphenols such as gallic acid and mangiferin found in mango seed kernels are able to chelate Cu(II) located in the center of the active site of tyrosinase. In silico studies revealed that these phenolics and L-DOPA occupy the same binding site surrounding the two-copper active site (Hering et al. 2023; Nithitanakool et al. 2009). Having ortho-dihydroxyphenols, phenolics in mango seed kernel share similar structural features with L-DOPA and hence act as competitive tyrosinase inhibitors that slow down the production of melanin (Maisuthisakul and Gordon 2009). Meanwhile, the effect of the production volume on the anti-tyrosinase activity of SPMK was found to be statistically not significant ($p > 0.05$). This suggests that the efficient extraction of polyphenols and their retention during the spray drying process support the feasibility of SPMK for larger-scale production.

Tyrosine inhibitory activity

Table 5: Half-maximal inhibitory activity (IC50) of the spray-dried phenolics and some tyrosinase inhibitor standards

Property	SPMK at varying production volumes			Ascorbic acid	Kojic Acid
	1800 mL	9000 mL	14400 mL		
IC_{50} (µg*)	6.91 ± 0.45^{c}	6.62 ± 0.24^{c}	6.72 ± 0.35^{c}	13.19 ± 0.20^{a}	2.94 ± 0.03^{d}

Different superscript indicates statistical significance ($p < 0.05$); analyzed using Welch's ANOVA with Games-Howell test. *Spray-dried phenolics are expressed in µg GAE.

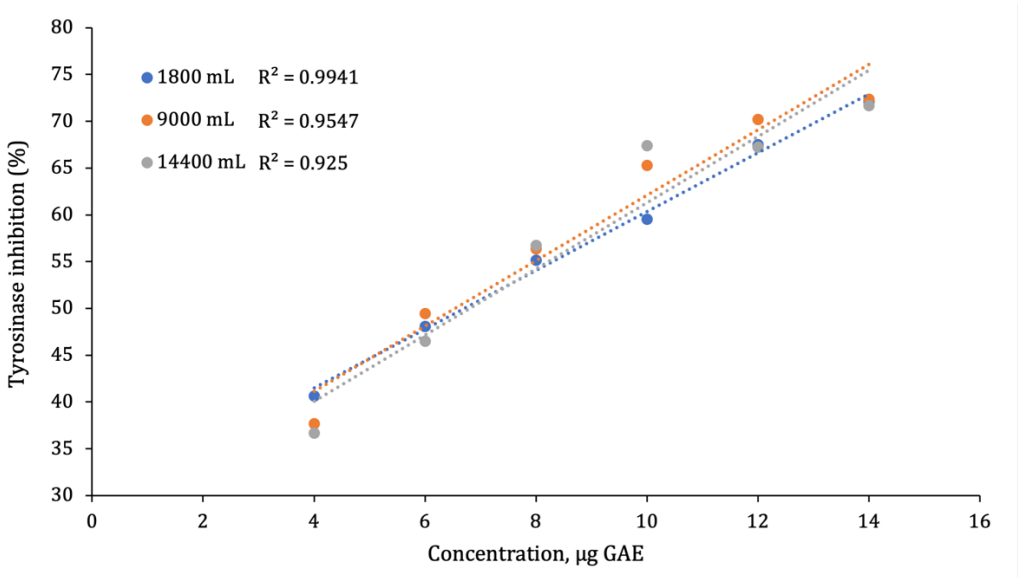

Figure 1: Inhibitory activity against tyrosinase of spray-dried phenolics extracted from mango kernels. The linearity constant showed concentration-dependent activity of the encapsulated phenolic compounds.

In addition, comparative analysis showed that SPMK exhibits comparable tyrosinase inhibitory activity compared to commercially used antioxidants. Table 5 shows that the phenolic powders are more potent tyrosinase inhibitors than ascorbic acid (IC_{50} = 13.19 µg) but less potent compared to kojic acid (IC_{50} = 2.94 µg). While kojic acid has shown greater potency, it has been associated with safety concerns such as skin irritation and photodamaging effects. The Cosmetic Ingredient Review (CIR) Expert Panel and the European Commission's Scientific Committee on Consumer Products (SCCP) found that the use of kojic acid beyond a concentration of 1.0 % in skin care formulations poses a risk to human health (Bernauer et al. 2022; Burnett et al. 2010). This safety concern associated with kojic acid and other synthetic activities has led consumers to seek natural and 'organic' cosmetic ingredients as alternatives (Statista, 2023). As for mango seed kernel extracts, several animal and clinical studies suggest that the extract is safe for use in cosmetic and skincare products. Mandawgade and Patravale (2008) reported that the use of mango butter, derived from mango seed kernels, in formulated foot care cream (FCC) did not result in irritation and sensitization both in their animal model testing and in their clinical testing. Similarly, Khan et al. (2022) reported that no adverse effects were observed when they applied a formulated topical emulsion loaded with mango peel and kernel extracts on the cheeks of human volunteers. Although it is worth noting that allergic reactions may vary depending on skin types and thus must be taken into account in formulations.

CONCLUSION

Our previous efforts have led to the standardization of spray-dried phenolics extracted from mango seed kernels which is now being harnessed for pilot-scale and industrial production. In this study, we explored the effect of 5-fold and 8-fold increase in production in terms of their physicochemical and antioxidant properties and dermal enzyme inhibitory activity. We found that increasing production volume has a minor effect on the physical properties and bioactivities of the spray-dried phenolics and can be improved by re-optimizing production conditions, particularly on the drying process. Through several in vitro assays, it was determined that phenolic powders have superior antioxidant activity when compared against ascorbic acid, indicating its potential for offsetting oxidative stress. The results also showed inhibitory activity against tyrosinase, highlighting its promising potential application as a skin-lightening agent.

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CONFLICT OF INTEREST

The authors of this research declare that there is no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

MKNA served as the principal investigator of the study, responsible for the conceptualization of the experiments and process optimization. JKAV, a research associate, handled the characterization of the SPMK and interpretations of the data. Both MKNA and JKAV contributed to the writing and editing of this paper. LSNA, another research associate, conducted production trial runs. ABS is the main technology inventor and acted as consultant during the conduct of the study. FMZT, another consultant, assisted in the conceptualization of the study. While CPD, research associate, assisted in all experiments throughout the study period.

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