Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00168

Comparison of conventional and supercritical CO_2 extraction techniques of *Curcuma longa* L. oleoresin and optimization of the spray drying process

Maritza BARRIGA-SÁNCHEZ[1]*, Gloria SANCHEZ-GONZALES[1], Miguel CUEVA-MARTÍNEZ[2], Erika Amalia MEDINA-CÁCERES[2], Jorge VILLACRES-VALLEJO[3]

Abstract

Turmeric (*Curcuma longa*) is used as fresh turmeric and flour in Loreto, Peru. However, there are still no studies on the characterization and utilization of oleoresin as an input for the food industry. The objectives of this study were to compare conventional and supercritical CO_2 extraction techniques of turmeric oleoresin and optimize the microencapsulation process through spray drying. Yield, curcuminoid content, total phenolic content (TPC), and antioxidant capacity (AC) of oleoresins obtained with supercritical CO_2 and conventional extraction techniques were compared. Spray drying process parameters for microencapsulation of oleoresin were optimized. The highest extraction yield of oleoresin was obtained with ethanol and methanol. However, these extracts had low values of total curcuminoids of 25.81% and 29.31%, respectively. Oleoresins extracted with supercritical CO_2 (SC-CO_2), ethanol, and acetone showed high contents of TPC with 0.2697, 0.2602, and 0.2560 g GAE/g oleoresin, respectively, while oleoresin extracted with SC-CO_2 had the highest AC by ABTS (2491.96 µmol TE/g oleoresin). Optimum conditions of spray drying were obtained with 190°C and a feed flow of 3.3 mL/min. In conclusion, *Curcuma longa* oleoresin is a valuable nutritional source for potential use in the food industry.

Keywords: bisdemethoxycurcumin; curcumin; demethoxycurcumin; green technology; microencapsulation; response surface.

Practical Application: Turmeric oleoresin is a source of bioactive compounds such as curcuminoids that can be used to develop healthy foods. Extraction with supercritical fluids such as CO_2 and ethanol maximizes curcuminoid content in turmeric oleoresin. Microcapsules of oleoresin obtained by spray drying are a promising proposal for the food industry.

1 INTRODUCTION

Turmeric (*Curcuma longa*) from the Zingiberaceae family is an important medicinal plant whose rhizomes are commonly used in the Chinese daily diet and in South and East Asia (Sheu et al., 2021). It is also used as a dye due to its characteristic golden yellow color (Omosa et al., 2017) and as medicine and food (Maggi, 2022).

Turmeric stands out for its antimicrobial, anticancer, and anti-inflammatory properties (Elanthendral et al., 2021). Likewise, it is a source of antioxidant, antimutagenic, anticoagulant, and antimicrobial compounds (Amalraj et al., 2017), which is why it is universally considered one of the "wonder drugs of life" (Elanthendral et al., 2021). The yellow color of rhizomes is due to the presence of three curcuminoids that are curcumin (CCM), demethoxycurcumin, and bisdemethoxycurcumin (BMC). CCM, which is one of the curcuminoids found in the rhizome, has shown pharmacological activities like hepatoprotective effects in animals (Singh & Sharma, 2011). Thus, CCM could be one of the constituents responsible for the bioactivity properties of the rhizome (Omosa et al., 2017).

In Peru, turmeric is produced in Amazonas, San Martin, Huanuco, Junin, Ayacucho, Cusco, and Loreto. In 2019, Peru ranked 12th as the world supplier of fresh turmeric with an annual growth rate of 24% (Sierra y Selva Exportadora, 2020). However, at present, there are still no reports on curcuminoid content and antioxidant capacity (AC) of turmeric oleoresin from the Peruvian Amazon.

With a CCM content of 30–40% and a volatile oil content of 15–20%, oleoresin is one of the derived products obtained from turmeric. Oleoresin is generally used for cooking, in meats and certain processed products such as mustard preparations, pickles and seasoning formulas, frozen fish fillets, frozen hash browns, butter, and cheese (Chempakam & Parthasarathy, 2008). Several methods for obtaining oleoresins have been reported, such as the use of organic solvents, Soxhlet extraction, ultrasonic extraction, microwaves, subcritical water, and supercritical CO_2 (Munekata et al., 2021). Supercritical CO_2 has been described as a promising technique for oleoresin extraction from *C. longa* and *Curcuma amada* (Nagavekar and Singhal, 2019). This is due to the low viscosity and density of CO_2 that provide a favorable

Received 20 Sept., 2023.
Accepted 2 Nov., 2023.
[1]*Instituto Tecnológico de la Producción, Laboratorio de Compuestos Bioactivos, Callao, Peru.*
[2]*Instituto Tecnológico de la Producción, Laboratorio Fisicoquímico, Callao, Peru.*
[3]*Universidad Nacional de la Amazonía Peruana, Facultad de Agronomía, Jirón Nauta, Iquitos, Peru.*
*Corresponding author: mbarriga@itp.gob.pe
This document has an erratum: https://doi.org/10.5327/fst.00168ERRATUM

mass transport and solubility for the extraction of curcuminoids in *C. longa* L. (Widmann et al., 2022).

Several authors have described microencapsulation as one of the techniques to improve the stability and bioavailability of bioactive compounds. Bioactive compounds are part of the core that is surrounded by a layer of wall materials. This layer functions as a physical barrier that prevents molecular diffusion and chemical reactions of the encapsulated bioactive compounds which improve stability (Taguchi et al., 2016). Thus, there are several types of microencapsulation techniques, such as spray drying, spray cooling, extrusion, centrifugal extrusion, freeze drying, and molecular inclusion, among others. However, spray drying is the most commonly used technique because it is flexible, cost-effective, fast, reproducible, and easily scalable while producing high-quality microparticles (Lucas et al., 2020). Spray drying also utilizes various wall materials like β-cyclodextrin, which successfully masks the flavor of lipids (Böttcher et al., 2015) and may help to mask undesirable flavors of turmeric oleoresin microcapsules.

Consequently, the objective of this study was to compare yield, curcuminoids, total phenolic content (TPC), and AC of turmeric oleoresins obtained by conventional and non-conventional methods. In addition, this research aimed to optimize spray drying parameters for microencapsulation of turmeric oleoresin, obtained with supercritical CO_2, to improve its stability and increase microencapsulation yield.

2 MATERIALS AND METHODS

2.1 Curcuma

Plants of *C. longa* L., from the Zingiberaceae family, were identified by the Amazonian Herbarium (AMAZ) and registered with code AMAZ 42787. Rhizomes were sown in the Caserío Angel Cárdenas from San Juan, Maynas, Loreto (2'58.954" S, 73°25'46.047" W) at 132 m.a.s.l. The humid tropical climate of the area had a temperature range from 20 to 32°C and a rainfall of 2,500 mm/year (SENAMHI, 2022). In January 2022, 8 months after sowing, the plants with rhizomes at the phenological stage of flowering were harvested by digging up. Then, 250 kg were transferred to the Instituto de Medicina Tradicional (IMET) located in Iquitos (Loreto-Peru) where they were washed and sorted. Yellow and orange rhizomes with 5–8 cm length and 1.5–2 cm diameter were selected and disinfected with 2% sodium hypochlorite for 30 min. They were cut and dried in a drying chamber with a dehumidifier (25 Pint, Peru) at 30–35°C for 6 days until the moisture content reached < 0.1 g/g with a drying yield of 10%. Then, dried flakes were ground in an analytical mill (A 11 Basic, IKA, USA), passed through a 500-µm sieve, and retained in a 500-µm sieve (Retsch, Peru). Turmeric flour (TF) was vacuum packed in polyethylene bags, protected from light, and refrigerated at 5 ± 1°C until later use.

2.2 Chemical reagents

Folin Ciocalteu's phenol reagent (2N), monohydrated gallic acid (≥ 98.5%, ACS), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; ≥ 96%), potassium iodide solution (> 99.0%, ACS), acetic acid (≥ 99.7%, ACS), and demethoxycurcumin standard (≥ 95%, HPLC) were obtained from Sigma-Aldrich (Canada). CCM standard (≥ 95%) was obtained from Bio Basic (Canada), and BMC standard (≥ 98%, HPLC) was obtained from Toronto Research Chemicals (Canada). Sodium carbonate (≥ 99.9%), iron trichloride hexahydrate (ACS), calcium standard (in HNO_3 1000 mg of Ca in 1 L), acetone (≥ 99.8%, ACS), methanol (≥ 99.9%, ACS), chloroform (≥ 99.8%, ACS), and sodium thiosulfate (ACS) were purchased from Merck (USA). The reagent 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (98%) was purchased from Alfa Aesar (Germany), and ethanol (99.5%) was purchased from Scharlau (Spain). Deionized water was supplied by the Barnstead water purification system (Barnstead, Model D11911, Germany). Carbon dioxide 99.5% v/v liquefied gas and nitrogen atmosphere Ultrapure were purchased from Linde (Peru). Hydrochloric acid ultrapure reagent (32–35%) was obtained from J.T.Baker (Canada). Whey protein concentrate (80%) was obtained from Saputo (United States), β-cyclodextrin was obtained from Wacker (Germany), and guar gum was obtained from Shree Vijaylaxmi International (India).

2.3 Oleoresin extraction from turmeric flour

Oleoresin extraction was carried out in triplicate by supercritical and conventional extraction at the Instituto Tecnológico de la Producción (Callao, Peru).

Extraction with supercritical CO_2 and ethanol as co-solvent was performed with a multi-solvent extractor equipment model 2802.000 (Top industrie, Vaux le Pénil, France) as described by Barriga-Sánchez et al. (2022). Approximately, 25 ± 0.5 g of TF and five alternating layers of 5 mm glass beads (5 g) were filled into the extraction cell. The extraction process was performed at 350 bar, 65°C, CO_2 flow of 35 g/min, and an extraction time of 150 min with ethanol (30% v/w) as a modifier as described by Nagavekar and Singhal (2019). Subsequently, the collected ethanolic extract was evaporated to dryness in a rotary evaporator (Buchi, R-300, Germany). Traces of solvent were removed using nitrogen, and oleoresin was stored at -20°C until further analysis.

Conventional extractions were performed with absolute ethanol, methanol, and acetone as recommended by Bagchi (2012). A TF:solvent ratio of 1:5 (w:v) was used, vortexed for 1 min, placed in an ultrasonic bath (VWR Symphony, 97043-942, USA) for 25 min, and centrifuged (Orto Alresa, Digtor 21R, Spain) at 3700 rpm for 30 min at 15°C. The extract was filtered using Whatman No. 42 filter paper, and the residue was subjected to four additional extractions following the same procedure. In each of the extractions, the solvent was evaporated using a rotary evaporator (Buchi, R-300, Germany) at 40°C, and oleoresin was stored in Ultrapure nitrogen atmosphere at -19°C until further analysis.

Oleoresin yield was calculated according to Equation 1:

$$\text{Oleoresin yield (\%)} = \frac{w1}{w2} x100 \quad (1)$$

Where:

$w1$: the oleoresin weight obtained after extraction (g);

$w2$: the TF weight (g).

2.4 Characterization of oleoresin

2.4.1 Quantification of bisdemethoxycurcumin, dimethoxycurcumin, and curcumin

Quantification of BMC, CCM, and dimethoxycurcumin (DMC) was made as described by Ashraf et al. (2015), Cao et al. (2014), and Jude et al. (2018). High-resolution liquid chromatography was performed with a UHPLC system (Ultimate 3000 RS, Thermo Scientific, Germany) equipped with a binary pump, an autosampler, and a triple-quadrupole mass spectrometer (TSQ Altis, Thermo Scientific, Germany) fitted with an electrospray ionization source (H-ESI).

Analytes were separated with a Waters ACQUITY UPLC BEH C18 column (150 mm × 2.1 mm × 1.7 µm) equipped with a corresponding pre-column (5 mm × 2.1 mm × 1.7 µm) conditioned at 40°C. The mobile phase contained acetonitrile -10 mM ammonium formate (pH 5) in a ratio of 70:30 (v/v). Separation was achieved by isocratic mode with a flow rate of 250 µL/min using an injection volume of 1 µL with a total run of 5 min. Optimized MS conditions, as shown in Supplementary Table 1, were as follows: spray voltage -3,500 V, vaporizer temperature 350°C, and ion transfer tube temperature 325°C. Nitrogen (99% purity) was used as sheath gas, auxiliary gas, and sweep gas at flow rates of 60, 23, and 0 arbitrary units (Arb), respectively. Finally, Argon was used as a collision gas at 1.5 mTorr. Calibration standards of curcuminoids were prepared in the concentration range from 5 to 1,000 ppb in LC-MS grade methanol. Solutions were sonicated using an ultrasonic bath, filtered through a 0.22-µm pore size PTFE membrane, and stored at 2–8°C until further use.

For the quantification of curcuminoids, the solvent was removed from an aliquot of the sample under a stream of nitrogen. Then, the sample was reconstituted in LC-MS grade methyl alcohol and diluted to a final concentration of 200 ppb, sonicated, and filtered through a 0.22-µm pore size PTFE membrane. Following filtration, samples were injected as such into the UHPLC system. The quantification of curcuminoids was done by multiple reaction monitoring and expressed as g/100 g of turmeric oleoresin. Analysis was carried out in triplicate.

2.4.2 Total phenolic content

TPC was determined according to the Folin-Ciocalteu procedure described by Singleton et al. (1999) with modifications. Briefly, 71 µL of extract (0.05 g turmeric oleoresin + 2 mL of methanol) was combined with 71 µL of Folin Ciocalteu, 1,430 µL of 6% sodium carbonate (w/v), and 2,000 µL of deionized water. The mixture was allowed to stay in the dark for 1 h at room temperature. Reading was carried out at 750 nm using a spectrophotometer model Genesys 180 (Thermo Scientific, USA), and a curve was generated with standard solutions of 50, 100, 150, 200, and 400 mg/L. Results were expressed in g of gallic acid equivalent (GAE) per g of oleoresin. Analysis was carried out in triplicate.

Table 1. Components of turmeric oleoresin dispersion.

Components	Wet basis (%)	Dry basis (%)
Oleoresin	1.28	23.49
β-cyclodextrin	1.19	21.83
Whey protein	2.70	49.54
Guar gum	0.28	5.14
Distilled water	94.55	0.00

2.4.3 Antioxidant capacity

2.4.3.1 FRAP

AC of turmeric extracts was determined using FRAP assay as described by Benzie, and Strain (1996). An appropriate amount of diluted samples was added to FRAP reagent (acetate buffer pH 3.6, TPTZ (2,4,6-tripyridyl-s-triazine), and $FeCl_3$ $6H_2O$ in a ratio of 25: 2.5: 2.5. The mixture was incubated at 20°C for 30 min, and absorbance was measured with a Genesys 180 UV-VIS spectrophotometer (Thermo Scientific, USA) at 595 nm. A calibration curve was prepared using Trolox standard solutions of 50, 150, 300, 400, 500, and 600 µM. FRAP values were expressed as µmol TE per g of oleoresin. Analysis was performed in triplicate.

2.4.3.2 ABTS

The 2-2'-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging capacity test of oleoresin was performed according to Prior et al. (2005). Previously, oleoresin samples were diluted with methanol. Results were expressed as µmol of Trolox equivalent (TE) per gram of oleoresin using a reference curve with concentrations of 0.1, 0.5, 1.0, 1.5, and 2.0 mM Trolox in ethanol. Analysis was performed in triplicate.

2.5 Microencapsulation of turmeric oleoresin

2.5.1 Dispersion of turmeric oleoresin

Dispersion was prepared as shown in Table 1. Turmeric oleoresin extracted with SC-CO_2 was mixed with β-cyclodextrin for 2 min. Then, distilled water was added at 60°C and stirred well for 2 min. This was followed by the addition of whey protein diluted in water (1:3, w:v), homogenization for 1 min, and sonication of the mixture in an ultrasonic bath (UP400St, Hielscher Ultrasonics, Germany) at 24 kHz frequency using 70% amplitude and a pulse of 70% for 45 min in an ice-water bath. Afterward, guar gum:water (1:100, w:v) was added and agitated for 30 s in a mixer (Hamilton Beach, 730, China) of 60 Hz. The formulation contained oleoresin:wall material in a ratio of 1:3.25.

2.6 Zeta potential for dispersion of turmeric oleoresin

Zeta potential was determined to evaluate the stability of oleoresin dispersion before spray drying. It was assessed by dynamic light scattering (DLS) using a Brookhaven 90Plus analyzer (Brookhaven Instruments, USA) with a system of electrodes. Data were analyzed using the BIC Zeta Potential Analyzer software v. 5.20 (USA). Briefly, the electrode system was immersed in a 1 mM KCl solution for 30 min and then

removed. Separately, a transparent plastic cuvette containing 1 mL of sample was placed in the sample holder of equipment along with the electrode system. The zeta potential measurements were performed for duplicate samples with three readings for each of them.

2.7 Response surface experimental design to optimize spray drying parameters of oleoresin dispersion

Optimization of drying parameters was carried out using spray drying equipment (TP-S15II, Toption, China). Preliminary spray drying tests were conducted with condensation appearing in the drying chamber at 140 and 150°C. Thus, for the response surface methodology (central-composite design), the low-level drying temperature was set at 160°C, the maximum temperature was 195°C, and the feeding rate was as follows: low level, 10%, and high level, 12%, which were equivalent to 3.3 and 4 mL/min, respectively. For all experiments, 140 g of dispersion, a 0.50-mm nozzle, a spray time of 15 s, and a pressure of 2.2–2.8 bar were used. Response variables were microencapsulation yield (%R) and encapsulation efficiency (%EE). The experimental design is shown in Table 2.

2.7.1 Microencapsulation yield

It was calculated for each experiment, using a gravimetric technique, according to Equation 2:

$$\% R = \frac{weight\ of\ recovered\ solids}{dispersion\ solids} x100 \quad (2)$$

2.7.2 Encapsulation efficiency

It was determined as described by Guo et al. (2020), with some modifications, for each microencapsulated sample obtained according to the parameters listed in Table 2, using Equation 3:

$$\%EE = \frac{(Total\ curcuminoids - Surface\ curcuminoids)}{Total\ curcuminoids} x100 \quad (3)$$

To determine the surface curcuminoids, 0.02 g of microcapsules was weighed and dissolved in 1 mL of 25% ethanol (v/v). The mixture was vortexed for 15 s and then centrifuged at 10,000 rpm for 10 min at 8°C. The supernatant was recovered, and this procedure was repeated three times. All the recovered extracts were combined and brought to a final volume of 10 mL in a volumetric flask. The extract was then diluted to determine curcuminoids, which were expressed as g curcuminoids/100 g of microcapsules.

Table 2. Central composite design.

Run	Inlet air temperature (°C)	Feed flow rate (%)
1	160.0	10.0
2	195.0	10.0
3	160.0	12.0
4	195.0	12.0
5	152.8	11.0
6	202.2	11.0
7	177.5	9.6
8	177.5	12.4
9	177.5	11.0
10	177.5	11.0

Total curcuminoids were determined as follows: 0.02 g of microcapsules was weighed, and 800 µL of water was added. The mixture was vortexed for 1 min and sonicated at 50 Hz for 15 min in an ultrasonic bath (VW10, Memmert, Germany) to help break the microcapsule wall. Then, it was centrifuged at 10,000 rpm for 10 min at 8°C, and the supernatant was recovered. These steps were repeated five times, with water being replaced by 1 mL of 96% ethanol each time until the sample was completely decolorized. Finally, all the recovered extracts were brought to a final volume of 10 mL in a volumetric flask and diluted to determine curcuminoids. Results were expressed as g curcuminoids/100 g of microcapsules.

A calibration curve of CCM standard between 1 and 19 µg/mL using 96% ethanol was prepared, and readings were taken on a spectrophotometer (Thermo Scientific, Genesys 30, USA) at 425 nm. For the quantification of curcuminoids, an aliquot of the extracts obtained from the microcapsules was taken and diluted with 96% ethanol until the concentration fell within the range of the calibration curve.

2.8 Measurement of particle size

The morphology of optimized microencapsulated particles was analyzed using a scanning electron microscope (SEM) (FEI, INSPECT S50, Czech Republic). Microcapsules were placed on aluminum holders with double-sided adhesive carbon tape and coated with gold using the sputtering method (SPI Sputter Coater, SPI Supplies, PA, USA). SEM examinations were performed at 5.0 kV using a working distance of 10 mm. Three images were selected, and sizes of 420 particles were measured using the Image J software (version 1.8.0, Wayne Rasband, USA).

2.9 Proximate composition analysis

Proximate composition analysis of microcapsules was done according to the Food and Agriculture Organization of the United Nations (1986). Moisture content was estimated using the gravimetric method by drying the sample in an oven (Venticell Ecoline, Czech Republic). Ash content was determined by incinerating the dried sample overnight in a muffle furnace (Barnstead, Thermolyne, Model 48000, USA). Total nitrogen content was determined by the Kjeldahl method using an automated Kjeldatherm TZ block digester (Germany) and a distillation unit Buchi K-350 (Spain). Fat content was determined by the Soxhlet method using a Universal Extractor Buchi E-800 (Switzerland). All analyses were performed in duplicates.

2.10 Statistical analysis

Yield, curcuminoids, TPC, and AC of oleoresins were analyzed for significant differences using a one-way analysis of variance (ANOVA). Tukey's multiple comparisons of means

were determined at the 0.05 confidence level. The response surface methodology design was used to optimize the drying parameters. All analyses were carried out with Minitab v.19 (Minitab, USA). Data were reported as mean ± standard deviation and were calculated with Excel 2016 (Microsoft, USA).

3 RESULTS AND DISCUSSION

3.1 Effect of different extraction methods on oleoresin yield

Oleoresin exhibited the highest extraction yield with ethanol and methanol ($p > 0.05$) (Table 3). No significant differences were observed between SC-CO_2 and acetone ($p > 0.05$). Oleoresins obtained with acetone and SC-CO_2 showed the lowest yields of 21.44% and 21.21%, respectively. However, these values were higher than those reported in Jamaican turmeric oleoresin extracted from fresh samples in a reflux system with 95% ethanol at 80°C (Green et al., 2008). Similarly, high values were also determined by Chassagnez-Méndez et al. (2000) when using supercritical CO_2 and ethanol at 40°C with 250 bar. Yield differences could be due to the solvating power of supercritical CO_2 and ethanol mixture that depends on pressure and temperature. The density of the solvent increases when pressure increases and, as a result, higher extraction yields are obtained (Osorio-Tobón, 2020), which could explain the high yield results when high pressures are used.

Despite the low yield of oleoresins obtained with acetone and SC-CO_2, those showed a higher content of total curcuminoids. Acetone and SC-CO_2 would be more selective when extracting curcuminoids. As cited by Revathy et al. (2011), the behavior of acetone may indicate that it has a higher selective extraction of curcuminoids than ethanol.

Table 3. Yield of turmeric oleoresins.

Solvent	Yield (%)
Methanol	26.49 ± 0.52[a]
Acetone	21.44 ± 0.51[b]
Ethanol	25.43 ± 0.65[a]
SC-CO_2	21.21 ± 1.20[b]

Values are expressed as mean ± standard deviation ($n = 3$). Different superscript letters within the same column represent significant differences ($p < 0.05$) according to Tukey's test.

3.2 Effect of different extraction methods on demethoxycurcumin, curcumin, and bisdemethoxycurcumin content

Total curcuminoids in the oleoresins were in the range of 25.81–35.85%. The highest contents of BMC, DMC, CCM, and total curcuminoids were found in oleoresins extracted with acetone and SC-CO_2, and no significant differences were found between those methods ($p > 0.05$). All oleoresin extracts showed CCM values that were higher than DMC and BMC values (Table 4).

Oleoresins obtained with acetone and SC-CO_2 showed the highest content of total and individual curcuminoids. Similar results were found by Revathy et al. (2011) and Verma and Jain (2011) in crude extracts of *C. longa* obtained with acetone when compared with ethanol, which demonstrates the importance of the solvent in the extraction of curcuminoids (Binello et al., 2020). Also, other authors reported a maximum oleoresin yield of 35.37 mg/g and curcuminoids of 0.187 mg/g when using supercritical CO_2 and ethanol at 350 bar (Nagavekar and Singhal, 2019), which were lower than the values reported in this study.

Among the curcuminoids under study, CCM was found in the highest amount, which agrees with other authors who reported CCM values of 46.45–67.31%, DMC of 11.47–23.81%, and BMC of 5.97–13.88% in crude extracts of turmeric from Thailand (Pothitirat & Gritsanapan, 2005).

3.3 Effect of different extraction methods on TPC and AC

The lowest TPC was determined in the oleoresin extracted with methanol (0.20 g GAE/g) (Table 5). No significant differences were found between the TPC of oleoresins extracted with acetone, ethanol, and SC-CO_2 ($p > 0.05$), which were in the range of 0.26–0.27 g GAE/g. Those values were higher than 0.20 mg GAE/g reported in commercial turmeric oleoresin from India (Nampoothiri et al., 2012).

Table 4. BMC, DMC, and CCM (g/100 g) in *Curcuma longa* L. oleoresins obtained by conventional methods and SC-CO_2.

Solvents	BMC (%)	DMC (%)	CCM (%)	Total curcuminoids (%)
Acetone	5.98 ± 0.32[a]	5.34 ± 0.32[a]	24.53 ± 1.33[a]	35.85 ± 1.97[a]
SC-CO_2	5.51 ± 0.43[ab]	5.00 ± 0.34[ab]	22.92 ± 0.93[a]	33.43 ± 1.70[a]
Methanol	5.02 ± 0.20[bc]	4.43 ± 0.19[bc]	19.86 ± 0.90[b]	29.31 ± 1.20[b]
Ethanol	4.38 ± 0.08[c]	3.82 ± 0.12[c]	17.60 ± 0.71[b]	25.81 ± 0.85[b]

Values are expressed as mean ± standard deviation ($n = 3$); Different superscript letters in the same column represent significant differences ($p < 0.05$) according to Tukey's test.

Table 5. TPC and AC of oleoresins from *Curcuma longa* L. obtained by conventional methods and SC-CO_2.

Solvents	TPC (g GAE/g)	ABTS (μmol TE/g oleoresin)	FRAP (μmol TE/g oleoresin)
Methanol	0.20 ± 0.01[b]	1797.70 ± 67.86[c]	752.75 ± 40.26[c]
Acetone	0.26 ± 0.01[a]	1732.32 ± 35.49[c]	1188.85 ± 62.87[a]
Ethanol	0.26 ± 0.05[a]	1995.49 ± 57.94[b]	1100.09 ± 24.00[a]
SC-CO_2	0.27 ± 0.01[a]	2491.96 ± 92.67[a]	971.60 ± 23.70[b]

Values are expressed as mean ± standard deviation ($n = 3$); Different superscript letters in the same column represent significant differences ($p < 0.05$) according to Tukey's test.

Despite low oleoresin yield when extracted with SC-CO_2 and acetone, they showed a higher AC by ABTS when using SCO-CO_2 than those extracted with organic solvents. On the contrary, the highest AC determined by FRAP corresponded to oleoresins extracted with acetone and ethanol (Table 5). Oleoresin obtained with methanol showed the lowest AC by ABTS and FRAP. Barzegar (2012) pointed out that mechanisms of hydrogen and electron transfer explain the antioxidant potential of CCM which are the mechanisms ABTS and FRAP methods are based on, respectively.

According to Singh et al. (2010), oleoresin extracted with ethanol has volatile compounds such as aromatic turmerone (9.6%), alpha santalene (7.8%), and alpha turmerone that contribute to the AC of oleoresin. This could suggest that a profile of volatile compounds may vary when using different solvents during the extraction process, and therefore this can cause variation in the AC values. On the contrary, CCM, the main active ingredient in turmeric, is mainly responsible for a high AC (Altir et al., 2021).

3.4 Zeta potential

Zeta potential is one of the important factors for evaluating the stability of dispersions (Fırtın et al., 2020). Absolute zeta potential values greater than 30 mV indicate higher stability of the emulsion, which can be improved by the use of ultrasound (Zhou et al., 2021). However, Honary and Zahir (2013) reported that values above 60 mV ensure excellent stability, values close to 20 mV provide only short-term stability, and values in the range of -5 to +5 mV indicate fast aggregation.

Also, positive and negative zeta potential measurements were observed at pH values lower and higher than 2, respectively (Fırtın et al., 2020). In this study, a value of -32.85 ± 1.87 mV was obtained at pH 6.06. This negative value could be attributed to the pH of the analysis, which indicates good stability. This result is close to the value of -32.2 ± 0.8 mV at pH 4.4 ± 0.04 reported by Fırtın et al. (2020) for dispersion of chia seed oil, maltodextrin, and gum Arabic. Additionally, Zamarioli et al. (2015) reported a zeta potential value of -35.3 to -21.2 mV, indicating a stable dispersion of curcuminoid concentrate with beeswax and Tween 80.

3.5 Optimization of spray drying parameters for oleoresin dispersion

3.5.1 Yield

Response surface regression analysis showed a significant linear model ($p < 0.05$) for yield, where inlet temperature (Ti) and feeding rate (FA) had a significant effect ($p < 0.05$) on yield (Supplementary Table 2).

In this study, a yield ranging from 49.94 to 59.27% was obtained, with an oleoresin:wall material ratio of 1:3.25. This yield fell within the range reported by Ipar et al. (2022), who reported a yield in the range of 48.10–69.02% using dairy protein, gum arabic, and maltodextrin as wall materials and an oleoresin:wall material ratio of 1:11. They also determined a curcuminoid content between 539.98 and 706.40 mg/100 g of microcapsules. On the contrary, Cano-Higuita et al. (2015) reported a lower yield which ranged from 23.97% to 57.68% using gum arabic, maltodextrin, and modified starch. Therefore, the type of material and equipment used could influence the yield. Generally, low yields between 20 and 50% are reported for laboratory-scale spray drying equipment (Ameri & Maa, 2006).

3.5.2 Encapsulation efficiency

Encapsulation efficiency is the percentage of solute that is successfully trapped in the encapsulated system (Woodhead & Hall, 2011). It represents the effectiveness of the wall material in protecting encapsulated curcuminoid compounds against losses or oxidation (Laokuldilok et al., 2016). In this study, the EE range was between 94.16 and 97.42% (Table 6) and showed a positive quadratic effect of Ti on the %EE values (Supplementary Table 2). The high %EE could be due to the aromatic ring of CCM being included in the cavity of the cyclodextrin, while the aromatic group at the other end of the molecule is not trapped in the cyclodextrin cavity (Laokuldilok et al., 2016).

Ferreira et al. (2019) obtained lower efficiency values that could be due to the difference in wall material and oleoresin: encapsulating material ratio, as well as the type of encapsulating material. They prepared dispersions in a ratio of 15 g of oleoresin with 100 g of encapsulating material (maltodextrin/gelatin) and evaluated drying air temperature and input flow of dispersion in the spray dryer. They reported %EE values, with different operating conditions, ranging from 3.54 to 77.18%. They also mentioned that the combination of a lower atomization air flow with a lower dispersion feed flow resulted in a higher %EE. In addition, they found that drying temperature had no significant effect on %EE. Similarly, Laokuldilok et al. (2016) studied oleoresin microencapsulation with other wall materials (e.g., whole rice flour and β-cyclodextrin) and reported %EE values of 46.72% and 97.11% in 1:1 and 1:14 core:encapsulating material ratio, respectively. They found a significant effect of dispersion feed flow on %EE, which aligns with the findings of this work.

Due to the lack of a significant encapsulation efficiency model (Supplementary Table 2), the optimization of the spray drying process was based solely on the higher yield criterion which resulted in the parameters of 190°C and 3.3 mL/min.

Table 6. Yield (%R) and encapsulation efficiency (%EE).

Run	Process yield (%)	Encapsulation efficiency (%)
1	57.31	95.93
2	59.27	96.80
3	49.94	96.98
4	57.60	96.92
5	53.82	97.42
6	58.02	96.52
7	57.46	96.92
8	55.62	95.35
9	57.12	94.75
10	56.63	94.16

3.6 Characterization of microcapsules

3.6.1 Proximal composition

Microcapsules had a moisture content of 1.55%. On a dry basis, microcapsules had 39.87% of protein, 1.58% of fat, and 1.58% of ash. The high percentage of protein in the microcapsules was mainly due to the contribution of whey protein (49.54% DB) as stated in the formulation for the dispersion of turmeric oleoresin.

3.6.2 Curcuminoids

Haiyee et al. (2009) mentioned that 20 mg of turmeric rhizome oleoresin requires 30 mg of β-cyclodextrin to form a soluble inclusion complex. In this study, 18.5 mg of β-cyclodextrin for 20 mg of oleoresin was used, and the total content of curcuminoids in the microencapsulated product was 6.78 g/100 g (BMC: 0.87 ± 0.01, DMC: 0.85 ± 0.01, and CCM: 5.07 ± 0.05 g/100 g). Aniesrani Delfiya et al. (2015) reported lower values that were in the range between 0.47 and 3.41 g/100 g while using maltodextrin and arabic gum as encapsulating materials. Laokuldilok et al. (2016) referred to a process of inclusion complexation between CCM and β-cyclodextrin through Van der Waals interactions, hydrogen bonding, and hydrophilic interaction. On the contrary, Zuanon et al. (2016) reported values of 26.6–34.2 g/100 g of total CCM using gelatin-collagen mixtures as encapsulating matrices.

3.6.3 Particle size using SEM

Contraction and deformation of atomized dried particles are related to temperature and water diffusion, as prolonged drying time causes the structure to deform, shrink, and collapse (Chen & Özkan, 2007). Microcapsules (Figure 1) were rounded and presented surfaces with concavities. Those concavities may be attributed to the drying air velocity, viscoelastic properties of the wall material, and contraction of the particles during the drying and cooling stages in the atomizer (Jafari et al., 2008, Sheu & Rosenberg, 1998). Even though the microcapsules had concavities, no cracks or pores were found on their surface. In addition, the microcapsules had similar characteristics to those obtained by Ferreira et al. (2019) which showed depressions, irregular concavities, and a tendency to form clusters. In this study, microcapsules had a particle size of 133.44 ± 19.97 μm that was smaller than the values reported by Ipar et al. (2022) (301.35–413.99 nm).

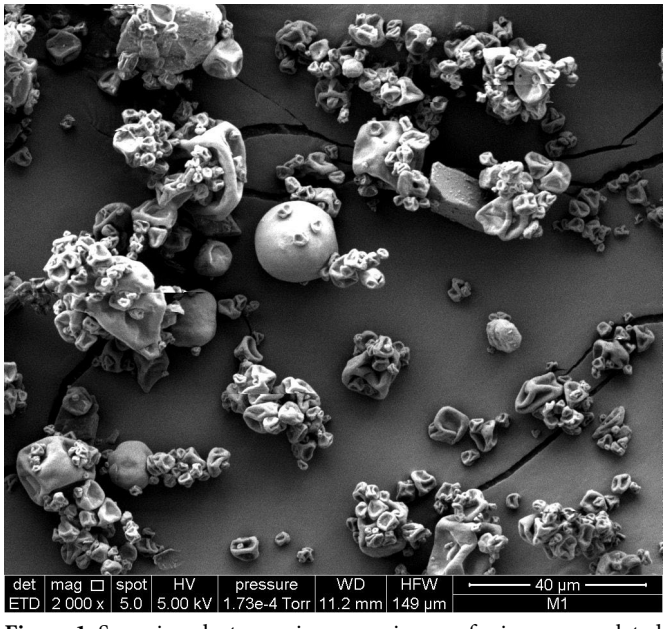

Figure 1. Scanning electron microscopy image of microencapsulated turmeric oleoresin dispersion.

4 CONCLUSION

Turmeric from the Peruvian Amazon is a valuable resource characterized by its high content of bioactive compounds, mainly curcuminoids, which can be utilized from the *C. longa* oleoresin. The highest content of curcuminoids was obtained with acetone and SC-CO2 extracts. Unlike conventional methods that use toxic solvents, extraction of turmeric oleoresin with $SC\text{-}CO_2$ is an environmentally friendly alternative. Using optimized parameters, spray drying of oleoresin achieved high EE values which indicated the effectiveness of the wall materials used. Thus, spray drying opens an opportunity to diversify the functional turmeric longa oleoresin market which is of great importance in the food and pharmaceutical industries.

ACKNOWLEDGEMENTS

This work was supported by PROCIENCIA (Programa Nacional de Investigación Científica y Estudios avanzados) project FONDECYT 046-2021.

REFERENCES

Altir, N. K. M., Ali, A. M. A., Gaafar, A.-R. Z., Qahtan, A. A., Abdel-Salam, E. M., Alshameri, A., Hodhod, M. S., & Almunqedhi, B. (2021). Phytochemical profile, in vitro antioxidant, and anti-protein denaturation activities of Curcuma longa L. rhizome and leaves. *Open Chemistry*, *19*, 945-952. https://doi.org/10.1515/chem-2021-0086

Amalraj, A., Pius, A., Gopi, S., & Gopi, S. (2017). Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives: A review. *Journal of Traditional and Complementary Medicine*, *7*(2), 205-233. https://doi.org/10.1016/j.jtcme.2016.05.005

Ameri, M., & Maa, Y.-F. (2006). Spray Drying of Biopharmaceuticals: Stability and Process Considerations. *Drying Technology*, *24*(6), 763-768. https://doi.org/10.1080/03602550600685275

Aniesrani Delfiya, D. S., Thangavel, K., Natarajan, N., Kasthuri, R., & Kailappan, R. (2015). Microencapsulation of turmeric oleoresin by spray drying and in vitro release studies of microcapsules. *Journal of Food Process Engineering*, *38*(1), 37-48. https://doi.org/10.1111/jfpe.12124

Ashraf, K., Mujeeb, M., Ahmad, A., Ahmad, N., & Amir, M. (2015). Determination of Curcuminoids in Curcuma longa Linn. By UPLC/Q-TOF-MS: An Application in Turmeric Cultivation. *Journal of Chromatography Science*, *53*(8), 1346-1352. https://doi.org/10.1093/chromsci/bmv023

Bagchi, A. (2012). Extraction of Curcumin. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, *1*(3), 1-16.

Barriga-Sánchez, M., Hiparraguirre, H. C., & Rosales-Hartshorn, M. (2022). Chemical composition and mineral content of Black Borgoña (Vitis labrusca L.) grapes, pomace and seeds, and effects of conventional and non-conventional extraction methods on their antioxidant properties. *Food Science and Technology*, *42*, e120021. https://doi.org/10.1590/fst.120021

Barzegar, A. (2012). The role of electron-transfer and H-atom donation on the superb antioxidant activity and free radical reaction of curcumin. *Food Chemistry*, *135*(3), 1369-1376. https://doi.org/10.1016/j.foodchem.2012.05.070

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, *239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Binello, A., Grillo, G., Barge, A., Allegrini, P., Ciceri, D., & Cravotto, G. (2020). A Cross-Flow Ultrasound-Assisted Extraction of Curcuminoids from Curcuma longa L.: Process Design to Avoid Degradation. *Foods*, *9*(6), 743. https://doi.org/10.3390/foods9060743

Böttcher, S., Steinhäuser, U., & Drusch, S. (2015). Off-flavour masking of secondary lipid oxidation products by pea dextrin. *Food Chemistry*, *169*, 492-498. https://doi.org/10.1016/j.foodchem.2014.05.006

Cano-Higuita, D. M., Vélez, H. A. V., & Telis, V. R. N. (2015). Microencapsulation of turmeric oleoresin in binary and ternary blends of gum Arabic, maltodextrin and modified starch. *Ciência e Agrotecnologia*, *39*(2), 173-182. https://doi.org/10.1590/S1413-70542015000300012

Cao, Y., Xu, R. X., & Liu, Z. (2014). A high-throughput quantification method of curcuminoids and curcumin metabolites in human plasma via high-performance liquid chromatography/tandem mass spectrometry. *Journal of Chromatography B*, *949-950*, 70-78. https://doi.org/10.1016/j.jchromb.2013.12.039

Chassagnez-Méndez, A. L., Machado, N. T., Araujo, M. E., Maia, J. G., & Meireles, M. A. (2000). Supercritical CO_2 extraction of curcumins and essential oil from the rhizomes of turmeric (*Curcuma longa* L.). Industrial & Engineering Chemistry Research, *39*(12), 4729-4733. https://doi.org/10.1021/ie000171c

Chempakam, B., & Parthasarathy, V. A. (2008). Turmeric. In V. A. Parthasarathy, B. Chempakam, & T. J. Zachariah (eds.). *Chemistry of spices* (pp. 97-123). CABI Publishing.

Chen, X. D., & Özkan, N. (2007). Stickiness, Functionality, and Microstructure of Food Powders. *Drying Technology*, *25*(6), 959-969. https://doi.org/10.1080/07373930701397400

Elanthendral, G., Shobana, N., Meena, R., Prakash, P., & Samrot, A. V. (2021). Utilizing pharmacological properties of polyphenolic curcumin in nanotechnology. *Biocatalysis and Agricultural Biotechnology*, *38*, 102212. https://doi.org/10.1016/j.bcab.2021.102212

Ferreira, S., Malacrida, C. R., & Nicoletti, V. R. (2019). Influence of emulsification methods and spray drying parameters on the microencapsulation of turmeric oleoresin. *Emirates Journal of Food and Agriculture*, *31*(7), 491-500. https://doi.org/10.9755/ejfa.2019.v31.i7.1968

Fırtın, B., Yenipazar, H., Saygün, A., & Şahin-Yeşilçubuk, N. (2020). Encapsulation of chia seed oil with curcumin and investigation of release behaivour & antioxidant properties of microcapsules during in vitro digestion studies. *LWT*, *134*, 109947. https://doi.org/10.1016/j.lwt.2020.109947

Food and Agriculture Organization of the United Nations (1986). Compositional analysis methods. In P. G. Martind (ed.). *Manual of food quality control* (p. 202-236). Food and Agriculture Organization of the United Nations.

Green, C. E., Hibbert, S. L., Bailey-Shaw, Y. A., Williams, L. A. D., Michell, S., & Garraway, E. (2008). Extraction, processing, and storage effects on curcuminoids and oleoresin yields from Curcuma longa L. grown in Jamaica. *Journal of Agriculture and Food Chemistry*, *56*(10), 3664-3670. https://doi.org/10.1021/jf073105v

Guo, J., Li, P., Kong, L., Xu, B. (2020). Microencapsulation of curcumin by spray drying and freeze drying. *LWT*, *132*, 109892. https://doi.org/10.1016/j.lwt.2020.109892

Haiyee, Z. A., Saim, N., Said, M., Illias, R. M., Mustapha, W. A. W., & Hassan, O. (2009). Characterization of cyclodextrin complexes with turmeric oleoresin. *Food Chemistry*, *114*(2), 459-465. https://doi.org/10.1016/j.foodchem.2008.09.072

Honary, S., & Zahir, F. (2013). Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 1). *Tropical Journal of Pharmaceutical Research*, *12*, 255-264. https://doi.org/10.4314/tjpr.v12i2.19

Ipar, V. S., Singhal, R. S., & Devarajan, P. V. (2022). An innovative approach using microencapsulated turmeric oleoresin to develop ready-to-use turmeric milk powder with enhanced oral bioavailability. *Food Chemistry*, *373*(Part A), 131400. https://doi.org/10.1016/j.foodchem.2021.131400

Jafari, S. M., Assadpoor, E., He, Y., & Bhandari, B. (2008). Encapsulation Efficiency of Food Flavours and Oils during Spray Drying. *Drying Technology*, *26*(7), 816-835. https://doi.org/10.1080/07373930802135972

Jude, S., Amalraj, A., Kunnumakkara, A. B., Divya, C., Löffler, B.-M., Gopi, S. (2018). Development of Validated Methods and Quantification of Curcuminoids and Curcumin Metabolites and Their Pharmacokinetic Study of Oral Administration of Complete Natural Turmeric Formulation (Cureit™) in Human Plasma via UPLC/ESI-Q-TOF-MS Spectrometry. *Molecules*, *23*(10), 2415. https://doi.org/10.3390/molecules23102415

Laokuldilok, N., Thakeow, P., Kopermsub, P., & Utama-ang, N. (2016). Optimisation of microencapsulation of turmeric extract for masking flavour. *Food Chemistry*, *194*, 695-704. https://doi.org/10.1016/j.foodchem.2015.07.150

Lucas, J., Ralaivao, M., Estevinho, B. N., Rocha, F. (2020). A new approach for the microencapsulation of curcumin by a spray drying method, in order to value food products. *Powder Technology*, *362*, 428-435. https://doi.org/10.1016/j.powtec.2019.11.095

Maggi, F. (2022). Antioxidants effects in health. The bright and the dark side. In S. M. Nabavi, & A. S. S. Silva (eds.). *Turmeric* (pp. 448-460). Elsevier.

Munekata, P. E. S., Pateiro, M., Zhang, W., Dominguez, R., Xing, L., Movilla, E., & Lorenzo, J. M. (2021). Health benefits, extraction and development of functional foods with curcuminoids. Journal of Functional Foods, 79, 104392. https://doi.org/10.1016/j.jff.2021.104392

Nagavekar, N., & Singhal, R. S. (2019). Supercritical fluid extraction of Curcuma longa and Curcuma amada oleoresin: Optimization of extraction conditions, extract profiling, and comparison of bioactivities. *Industrial Crops and Products*, *134*, 134-145. https://doi.org/10.1016/j.indcrop.2019.03.061

Nampoothiri, S. V., Lekshmi, P. C., Venugopalan, V. V., & Menon, A. N. (2012). Antidiabetic and antioxidant potentials of spent turmeric oleoresin, a by-product from curcumin production industry. *Asian Pacific Journal of Tropical Disease*, *2*(Suppl. 1), S169-S172. https://doi.org/10.1016/S2222-1808(12)60146-7

Omosa, L. K., Midiwo, J. O., & Kuete, V. (2017). Curcuma longa. *Medicinal Spices and Vegetables from Africa*, 425-435. https://doi.org/10.1016/B978-0-12-809286-6.00019-4

Osorio-Tobón, J. F. (2020). Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *Journal of Food Science and Technology*, *57*, 4299-4315. https://doi.org/10.1007/s13197-020-04433-2

Pothitirat, W., & Gritsanapan, W. (2005). Quantitative Analysis of Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin in the Crude Curcuminoid Extract from Curcuma longa in Thailand by TLC- Densitometry. *Mahidol University Journal of Pharmaceutical Sciences*, *32*, 23-30.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agriculture and Food Chemistry*, *53*(10), 4290-4302. https://doi.org/10.1021/jf0502698

Revathy, S., Elumalai, S., Merina, B., & Antony, B. (2011). Isolation, Purification and Identification of Curcuminoids from Turmeric (Curcuma longa L.) by Column Chromatography. *Journal of Experimental Science*, *2*(7), 21-25.

Servicio Nacional de Meteorología e Hidrología del Perú (SENAMHI). (2022). *Datos Hidrometeorológicos a nivel nacional*. SENAMHI. Retrieved from https://www.senamhi.gob.pe/?p=estaciones

Sheu, S.-C., Wu, Y.-C., Lien, Y.-Y., & Lee, M.-S. (2021). Specific, sensitive and rapid Curcuma longa turmeric powder authentication in commercial food using loop-mediated isothermal nucleic acid amplification. *Saudi Journal of Biological Sciences*, *28*(10), 5931-5936. https://doi.org/10.1016/j.sjbs.2021.06.057

Sheu, T.-Y., & Rosenberg, M. (1998). Microstructure of microcapsules consisting of whey proteins and carbohydrates. *Journal of Food Science*, *63*(3), 491-494. https://doi.org/10.1111/j.1365-2621.1998.tb15770.x

Sierra y Selva Exportadora (2020). *Oportunidades Comerciales - Jengibre y Cúrcuma*. Sierra y Selva Exportadora. Retrieved from https://www.gob.pe/institucion/sse/informes-publicaciones/1368938-oportunidades-comerciales-jengibre-y-curcuma-2020

Singh, G., Kapoor, I. P. S., Singh, P., Heluani, C., De Lampasona, M. P., & Catalan, C. (2010). Comparative study of chemical composition and antioxidant activity of fresh and dry rhizomes of turmeric (Curcuma longa Linn.). *Food and Chemical Toxicology*, *48*(4), 1026-1031. https://doi.org/10.1016/j.fct.2010.01.015

Singh, R., & Sharma, P. (2011). Hepatoprotective effect of curcumin on lindane-induced oxidative stress in male Wistar rats. *Toxicology International*, *18*, 124-129. https://doi.org/10.4103/0971-6580.84264

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In L. Packer (ed.). *Methods in enzymology* (pp. 152-178). Academic Press. https://doi.org/10.1016/S0076-6879(99)99017-1

Taguchi, Y., Saito, N., Uchida, A., & Tanaka, M. (2016). Preparation of Thermosensitive microcapsules containing water soluble powder by melting dispersion cooling method. *Journal of Encapsulation and Adsorption Science*, *6*(3), 57-69. https://doi.org/10.4236/jeas.2016.63006

Verma, S. C., & Jain, C. L. (2011). Solvent polarity based microwave-assisted extraction of curcuminoids from curcuma longa L. Rhizome and their quantitative determination by HPLC-PDA Method. *International Journal of Pharmaceutical Research*, *3*(4), 41-47.

Widmann, A.-K., Wahl, M. A., Kammerer, D. R., & Daniels, R. (2022). Supercritical Fluid Extraction with CO2 of Curcuma longa L. in Comparison to Conventional Solvent Extraction. *Pharmaceutics*, *14*(9), 1943. https://doi.org/10.3390/pharmaceutics14091943

Woodhead, J. L., & Hall, C. K. (2011). Encapsulation Efficiency and Micellar Structure of Solute-Carrying Block Copolymer Nanoparticles. *Macromolecules*, *44*(13), 5443-5451. https://doi.org/10.1021/ma102938g

Zamarioli, C. M., Martins, R. M., Carvalho, E. C., & Freitas, L. A. P. (2015). Nanoparticles containing curcuminoids (Curcuma longa): development of topical delivery formulation. *Revista Brasileira de Farmacognosia*, *25*(1), 53-60. https://doi.org/10.1016/j.bjp.2014.11.010

Zhou, L., Zhang, J., Xing, L., & Zhang, W. (2021). Applications and effects of ultrasound assisted emulsification in the production of food emulsions: A review. *Trends in Food Science and Technology*, *110*, 493-512. https://doi.org/10.1016/j.tifs.2021.02.008

Zuanon, L. A. C., Malacrida, C. R., & Telis, V. R. N. (2016). Effect of ultrasound on the stability of turmeric oleoresin microencapsulated in gelatin-collagen matrices. *Journal of Food Process Engineering*, *40*(2), e12360. https://doi.org/10.1111/jfpe.12360