Food Research International 50 (2013) 657–663

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Curcuminoid content and antioxidant activity in spray dried microparticles containing turmeric extract

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ARTICLE INFO

Article history:
Received 10 December 2010
Accepted 11 June 2011

Keywords:
Turmeric
Box Behnken design
Solubility
Morphology
DPPH

ABSTRACT

Curcuma longa L., also known as turmeric, is widely used as a food colorant and has been reported to have antioxidant, anti-inflammatory, anti-mutagenic and anti-cancer properties. The aim of this study was to evaluate the effects of the spray drying on curcuminoid and curcumin contents, antioxidant activity, process yield, the morphology and solubility of the microparticulated solid dispersion containing curcuma extract using a Box Behnken design. The microparticles were spherical in shape, and an increase in outlet temperature from 40 to 80 °C resulted in a significant increase in the yield of microparticles from 16 to 53%. The total curcuminoid content (17.15 to 19.57 mg/g), curcumin content (3.24 to 4.25 mg/g) and antioxidant activity (530.1 to 860.3 μg/mL) were also affected by the spray drying process. The solubility of curcuminoid from *C. longa* remarkably improved 100-fold in the microparticles, confirming the potential of the ternary solid dispersion technique to improve the dyeing and nutraceutical properties of these compounds. Furthermore, the microparticles were obtained using the spray drying process, can be easily scaled up.

1. Introduction

Curcuma longa L. (Zingiberaceae), also known as turmeric, is native to India, distributed throughout tropical and subtropical regions of the world and widely cultivated in Southeast Asia (Began, Goto, Kodama, & Hirose, 2000; Goel, Kunnumakkara, & Aggarwal, 2008). The rhizomes of *C. longa* provide a yellow, flavorful powder when dried and ground (Silva, Nelson, Drummond, Dufossé, & Glória, 2005) and have long been used in Chinese and Ayurvedic medicines (Joshi, Jain, & Sharma, 2009). Turmeric has also attracted considerable attention over the years due to its use in the food industry as a coloring agent (Aggarwal, Kumar, & Bharti, 2003). Unlike synthetic dyes such as tartrazine and carmoisine that may impair liver function and cause oxidative stress, many natural pigments are used not only as food coloring, but also as a substance that promotes health and well being by preventing or even healing diseases (Amin, Hameid, & Elsttar, 2010; Downham & Collins, 2000). *C. longa* has several proven pharmacological activities, including antioxidant (Jayaprakasha, Rao, & Sakariah, 2006), anti-inflammatory (Wang et al., 2008), anti-microbial (Wang, Lu, Wu, & Lv, 2009) and anti-tumor properties (Mitra, Chakrabarti, Banerji, Chatterjee, & Das, 2006), making it an interesting nutraceutical for chemoprevention purposes (Gosslau & Chen, 2004). These properties have been attributed to curcuminoids, which are a group of phenolic compounds composed mainly of curcumin, demethoxy curcumin and bis-demethoxy curcumin (Maheshwari, Singh, Gaddipati, & Srimal, 2006).

Despite these features, curcuminoids have poor stability and low aqueous solubility (Araújo, Teixeira, & Freitas, 2010). In fact, curcumin is practically insoluble in acidic solutions, unstable in solutions with a basic pH and breaks down easily, producing mainly ferulic acid, feruloylmethane and yellowbrown condensation products (Price & Buescher, 1997), which invalidates its use in pharmaceuticals and limits its use in food processing industries (Surojanametakul, Satmalee, Saengprakai, Siliwan, & Wattanasiritham, 2010; Wang et al., 2009).

To overcome this problem, *C. longa* can be converted to free flowing microparticles, improving the solubility of curcumin in acidic pH and maintaining its stability in aqueous media (Paradkar, Ambike, Jadhav, & Mahadik, 2004). Solid dispersion, SD, is a useful method for dispersing compounds in the molecular state in a carrier matrix (Takeuchi, Nagira, Yamamoto, & Kawashima, 2004). Numerous SD systems have been shown to improve the solubility and dissolution properties of compounds that have poor water solubility (Dhirendra, Lewis, Udupa, & Atin, 2009). According to Chiou and Riegelman (1971), SD is the dispersion of one or more active ingredients in an inert carrier or matrix at solid state. Hydrophilic polymers have been commonly used as carriers for preparing SD such as polyvinylpyrrolidone (PVP), polyethylene glycols (PEGs), polyoxylglycerides (Gelucires®), which have high physiological tolerance, and low toxicity (Dhirendra et al., 2009; Leuner & Dressman, 2000; Liu & Wang, 2007). The main methods

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0963-9969
doi:10.1016/j.foodres.2011.06.030

employed for SD preparation are the melting (fusion) and solvent method. The fusion method employs melting of the mixture of the drug and carrier in a temperature 10 °C above of the melting point of carrier and further solidification by cooling (Kamalakkannan, Puratchikody, Masilamani, & Senthilnathan, 2010). The solvent method consists on the solubilization of the drug and carrier in a volatile solvent that is later evaporated. Differences in solvent evaporation processes are related to the solvent evaporation procedure such as, spray-drying, freeze-drying and the use of supercritical fluids (Vasconcelos, Sarmento & Costa, 2007). There are several studies in the literature about the production of solid dispersions of poorly soluble drugs. Liu and Wang (2007) improved dissolution of oleanolic acid with the aid of polyvinylpyrrolidone and polysorbate 80 ternary solid dispersions. Rajarajan, Baby, Ramesh, and Singh (2009) enhanced dissolution rate of itraconazole by preparation of spray dried ternary solid dispersion using a mixture of polyethyleneglycol 6000 and hydroxypropylmethylcellulose. Lastly, the antifungal drug griseofulvin also had its dissolution rate enhanced when formulated with hydroxypropyl methylcellulose acetate succinate (HPMCAS) and poly[N-(2-hydroxypropyl) methacrylate] (PHPMA) (Al-Obaidi & Buckton, 2009).

The development of microparticulated solid dispersions using a spray drying technique is an interesting method for improving the stability and solubility of compounds (Araújo et al., 2010; Vasconcelos, Sarmento, & Costa, 2007). Spray drying gives spherical particles that are small in size and that have narrow distributions, and the drying is completed in a single step (Takeuchi et al., 2004). Spray drying is commonly used in the food industry due to its relatively low cost, its high productivity, the increased microbiological stability of food and phytopharmaceutical products and the diminished risk of chemical and/or biological degradations (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Besides, spray drying has been used to produce biomaterials and biopharmaceutical products (Teixeira, Teixeira, & Freitas, 2011).

The study aimed at optimization of spray drying process for the production of solid dispersion microparticles from extract of *C. longa* by Box Behnken design and subsequently evaluates the effect of spray drying on curcuminoids, their solubility, and antioxidant activity.

2. Material and methods

2.1. Material

Rhizomes of *C. longa* were purchased from the Brazilian market (YOD do Brasil Ltda, São Paulo) for extraction. Analytical-grade curcumin and 2,2-diphenyl-1-picrylhydrazyl were purchased from Sigma Inc. (Sigma-Aldrich Corp., St. Louis, USA). Colloidal silicon dioxide Aerosil® 200 (Evonik Ind AG, Germany) and polyvinylpyrrolidone (PVP K30) were supplied by Henrifarma Ltda (São Paulo, SP, Brazil). The mobile phases used were HPLC-grade acetonitrile and hydrochloric acid supplied by Merck (Darmstadt, Germany) and Synth (Sao Paulo, Brazil), respectively.

2.2. Methods

2.2.1. Extraction process

The best conditions for extraction of *C. longa* were evaluated by preliminary assays and led us to an ultrasonically extraction of three grams of ground *C. longa* rhizomes in 50 mL ethanol:water (7:3) solution using an ultrasound generation probe, model DE S 500 from UNIQUE (Indaiatuba, SP, Brazil), at 20 kHz and 300 W for 5 min. Extracts were filtered using a 0.45 μm filter.

The extract obtained from turmeric powder was assayed for total solids content (TSC) and total curcuminoid content. The TSC in the extract was determined using the gravimetric method. Aliquots of each experiment (5.0 mL) were weighed in petri-dishes and dried in a circulating air oven (37 ± 1 °C) to constant weight. The total curcuminoid content in the extract was determined by a UV/VIS spectrophotometer (Model 330W, Camspec, Garforth, UK) at 425 nm using a standard curve from analytical-grade curcumin ranging from 1.0 to 8.0 μg/mL with a correlation coefficient (R^2) of 0.9984 and the regression equation is given by $y = 0.1102x + 0.0254$.

2.2.2. Preparation of microparticles by spray drying

The experiments were carried out following a Box Behnken design with three factors (Table 1). The first factor was the outlet temperature (T) of the drying air, which is expected to be related to active loss during the drying. The second factor was the ratio (w/w) of colloidal silicon dioxide to the mixture of *C. longa* extract plus polyvinylpyrrolidone, or AM, defined by Eq. (1). The silicon dioxide is used to improve pharmaceutical powders flowability and also to decrease material stickiness (Schonherr & Riede, 1994).

$$\mathrm{AM} = \frac{\text{mass colloidal silicon dioxide}}{\text{mass PVP} + \text{mass } C.\ longa \text{ extract}}. \quad (1)$$

The third factor was the proportion (w/w) of polyvinylpyrrolidone (PVP K30) and *C. longa* extract, or PC, which is expected to be related to the enhanced solubility of curcuminoids, defined by Eq. (2).

$$\mathrm{PC} = \frac{\text{mass PVP}}{\text{mass } C.\ longa \text{ extract}}. \quad (2)$$

The experimental factorial design with three levels and three factors allows the determination of linear, quadratic and interactive effects (Box, Hunter, & Hunter, 1978). The factors and their levels are shown in Table 1. The factors were decoded to allow the ANOVA analysis following the decoding rule shown in Eq. (3).

$$\text{Code variable} = \frac{(\text{uncoded value} - 0.5 \times (\text{high value} + \text{low value}))}{0.5 \times (\text{high value} - \text{low value})}. \quad (3)$$

The analysis of variance for experimental data was performed by surface response methodology using the module Visual General Linear Model (VGLM) from Statistica 99 software (Statsoft Inc, USA).

Table 1
Box Behnken design.

Experiments	Factors		
	X_1 (T)	X_2 (AM)	X_3 (PC)
1	−1 (40)	−1 (0.3)	0 (3.0)
2	1 (80)	−1 (0.3)	0 (3.0)
3	−1 (40)	1 (0.7)	0 (3.0)
4	1 (80)	1 (0.7)	0 (3.0)
5	−1 (40)	0 (0.5)	−1 (1.0)
6	1 (80)	0 (0.5)	−1 (1.0)
7	−1 (40)	0 (0.5)	1 (5.0)
8	1 (80)	0 (0.5)	1 (5.0)
9	0 (60)	−1 (0.3)	−1 (1.0)
10	0 (60)	1 (0.7)	−1 (1.0)
11	0 (60)	−1 (0.3)	1 (5.0)
12	0 (60)	1 (0.7)	1 (5.0)
13	0 (60)	0 (0.5)	0 (3.0)
14	0 (60)	0 (0.5)	0 (3.0)
15	0 (60)	0 (0.5)	0 (3.0)

Notes: X_1, temperature (°C); X_2, ratio of colloidal silicon dioxide to the mixture of *C. longa* extract with polyvinylpyrrolidone (w/w); and X_3, proportion of polyvinylpyrrolidone (PVP K30) and *C. longa* extract (w/w).

The polynomial model shown in Eq. (4) was applied as the response function.

$$Y_i = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1X_2 + A_5X_1X_3 + A_6X_2X_3 + A_7X_1^2 + A_8X_2^2 + A_9X_3^2 \quad (4)$$

where Y_i = dependent variables: yield (Py), total curcuminoid content (TCC), curcumin content (CC) and antioxidant activity (IC_{50});

X_i factors studied; X_1 (T—temperature); X_2 (AM—ratio of silicon dioxide to *C. longa* extract and PVP, w/w); X_3 (PC—ratio of PVP to *C. longa* extract, w/w).
X_1X_2 represent the interaction term;
X_i^2 quadratic terms;
A_i polynomial coefficients.

The effects of the factors were considered significant when *p*-values were lower than 0.05.

Each suspension for spray drying was prepared by adding defined weights of colloidal silicon dioxide and PVP to 50 mL of the curcuma extracts according to the factorial design. The total solid content of the spray solution was 10%. This solution was kept on magnetic stirring for 10 min and sonicated for 5 min. During the drying process the solution was kept under magnetic stirring.

The suspensions were dried using a mini spray dryer model LM MSD 0.5 (Labmaq do Brasil Ltda, Ribeirão Preto, SP, Brazil) with the capacity of drying up to 0.5 L/h. The cylindrical drying chamber is made of borosilicate glass and has an internal diameter of 13 cm and is 51 cm in height. The outlet temperature can be controlled by a digital PID controller independently of air and liquid flow rates. The liquid atomization was performed with a pneumatic spray nozzle with orifice diameter of 1.2 mm, and the dryer was operated in cocurrent flow. The following sets of conditions were kept fixed for all experiments: suspension feed rate of 5.0 mL/min, atomization air pressure of 5.0 kgf/cm^2, drying air flow rate of 1.5 m^3/min and nozzle air flow of 40.0 L/min. The dried products were collected and kept in a desiccator at 25 °C and vacuum of 630 mmHg. The microparticles were thoroughly characterized for yield (Py), total curcuminoid content (TCC), curcumin content (CC), antioxidant activity and morphology. In addition, the solubility of the selected microparticles was evaluated and compared with the lyophilized extract.

2.2.3. Process yield (Py)

The microparticle yield was determined by the ratio of the weight of microparticles collected at dryer exit and the initial weight of dispersion taken for drying. The results were calculated as the percentage ratio of the final mass of microparticles to the initial mass of raw material (dry basis; %w/w) using the following definition (Eq. (5)):

$$\text{Py}(\%) = \frac{\text{mass of microparticles}}{\text{mass of raw material}} \times 100. \quad (5)$$

2.2.4. Scanning electron microscopy (SEM)

The morphological characteristics of the microparticles were observed by SEM using a SS-550 microscope (Shimadzu, Kyoto, Japan). Prior to SEM, the samples were coated with gold under an argon atmosphere using a sputter coater Bal-Tec SCD 050 (Fürstemtum Liechtenstein). Photomicrographs were taken at an acceleration voltage of 20.0 kV.

2.2.5. Total curcuminoid content (TCC)

The TCC was determined according to Rubesh, Ram, Venkateshwar, Duganath, and Kumanam (2010) with modification using a standard curve of analytical-grade curcumin. Concentrations of 1.0, 2.5, 5.0, 6.5 and 8.0 μg/mL of curcumin in ethanol were measured at a wavelength of 425 nm by a UV/VIS spectrophotometer (Model 330W, Camspec, Garforth, UK), and the resulting curve had a correlation coefficient (R^2) of 0.9984 and the regression equation is given by $y = 0.1102x + 0.0254$. Each sample was solubilized in ethanol and properly diluted. Experiments were performed in triplicate. The values of TCC were expressed in mg TCC/g dry weight of *C. longa*.

2.2.6. Determination of curcumin content (CC)

The quantification of curcumin in microparticles was performed using Shimadzu LC-10AT High-performance Liquid Chromatography (HPLC) coupled to a SPD-10A UV/VIS detector and a C-R6A integrator. The mobile phase was 1.0% acetonitrile:aqueous citric acid (1:1) with a flow rate of 1.0 mL/min on a CN column. The UV/VIS detector was set to a wavelength of 425 nm. The samples were injected in a Rheodyne injector with a loop of 20.0 μL. The chromatographic peaks were identified by comparison with the retention times of standard curcumin (Sigma-Aldrich Brasil Ltda, São Paulo, Brazil). The calibration curve was prepared using curcumin standards at concentrations of 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 μg/mL in methanol with a correlation coefficient (R^2) of 0.9992 and the regression equation is given by $y = 141708x + 3500.6$. The injections were made in triplicate. The content of curcumin in the extract was calculated using the standard curve. The values of CC were expressed in mg CC/g dry weight of *C. longa*.

2.2.7. TCC solubility

Solubility studies for each experiment were performed according to the method described by Damian et al. (2000). An excess amount of each sample (20.0 mg) was weighed in test tubes containing 10 mL of acidic HCl solution (pH = 1.2). The samples were magnetically stirred inside a thermostatic air bath at 37 ± 1 °C for 48 h. Suspensions were filtered using 0.22 μm filters and analyzed with a UV/VIS spectrophotometer (Model 330W, Camspec, Garforth, UK) at 425 nm. Solubility studies were performed in triplicate. The concentration of soluble curcuminoids, TCC, was calculated using the calibration curve of curcumin.

2.2.8. Determination of antioxidant activity (DPPH• test)

Antioxidant activities were determined by the hydrogen-donating ability of stable free radical DPPH• (2,2-diphenylpicrylhydrazil) according to Georgetti, Casagrande, Vicentini, Verri, and Fonseca (2006). A blank was prepared using the reaction mixture (without samples and DPPH• solution), and a positive control (C^+) was prepared with 1.0 mL ethanol, 1.0 mL acetate buffer and 0.5 mL DPPH• (without samples). All measurements were performed in triplicate. The percent antioxidant inhibition was estimated using Eq. (6).

$$\%\ \text{inhibition} = 100 - \frac{A \times 100}{C^+} \quad (6)$$

where A = absorbance; C^+ = absorbance of positive control.

The percentages of inhibition were plotted against the concentrations (31 to 1000 μg/mL range) for each sample, and the concentration that corresponded to 50% inhibition of DPPH reduction was reported as the IC_{50} value.

3. Results and discussion

3.1. Total solids content and TCC in the C. longa extract

The extraction of ground roots of *C. longa* with an ethanol:water (7:3) solution was conducted using ultrasound-assisted extraction (UAE). The total solids content in the extract was 3.08%, with TCC of 165.9 μg/mL. This extract was used for the spray drying study.

Table 2
Results of dependent variables studied with predicted and actual response values.

	Py		TCC		CC		IC_{50}	
Run nr	Pred.	Actual	Pred.	Actual	Pred.	Actual	Pred.	Actual
1	30.0	29	15.0	19.16 ± 5.88	3.80	3.90	684.7	532.4 ± 7.11
2	46.5	49	15.0	18.18 ± 5.09	3.80	3.58	684.7	613.0 ± 19.86
3	23.8	24	15.0	17.93 ± 2.05	3.58	3.45	684.7	665.3 ± 26.39
4	40.0	38	15.0	18.22 ± 2.19	3.58	3.49	684.7	748.6 ± 22.25
5	33.0	32	15.5	18.92 ± 4.64	3.51	3.56	705.9	576.5 ± 62.53
6	50.0	53	15.5	17.70 ± 4.38	3.51	3.51	705.9	661.4 ± 12.12
7	14.4	16	15.5	18.49 ± 2.95	3.87	4.02	705.9	737.9 ± 9.24
8	31.0	27	15.5	18.76 ± 5.08	3.87	3.85	705.9	668.3 ± 20.66
9	49.4	48	17.0	18.21 ± 4.84	3.27	3.24	571.2	541.6 ± 8.91
10	43.0	42	17.0	19.57 ± 3.06	3.75	3.78	571.2	530.1 ± 10.81
11	30.6	30	17.0	18.68 ± 1.78	4.33	4.25	571.2	589.7 ± 13.57
12	24.4	27	17.0	18.27 ± 2.29	3.41	3.37	571.2	622.8 ± 40.22
13	40.0	38	17.0	17.15 ± 3.92	3.69	3.72	819.3	830.3 ± 71.38
14	40.0	42	17.0	17.37 ± 1.91	3.69	3.54	819.3	860.3 ± 36.86
15	40.0	40	17.0	17.81 ± 2.70	3.69	3.83	819.3	767.7 ± 44.73

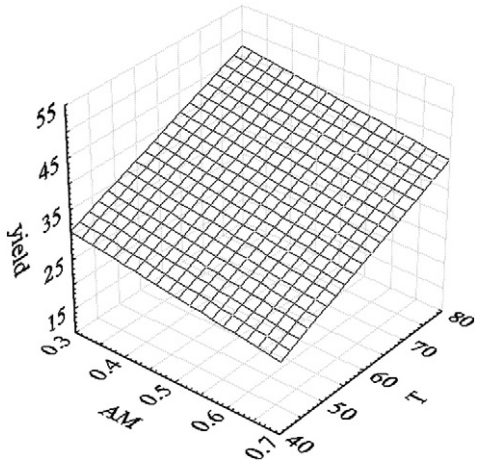

Fig. 1. Yield of microparticles as function of temperature (T) and the ratio of silicon to curcumin PVP mixture (AM).

3.2. Process yield (Py)

The Py for fifteen experiments based on the factorial design ranged from 16.0 to 53.0%. (Table 2). These results were satisfactory for a laboratory-scale spray apparatus because very low yields (<50%) are usually reported in spray dryers with a capacity of 1.0 L/h (Ameri & Maa, 2006). The analysis of variance by the response surface methodology showed that the decrease of PC and the nonlinear increase in the outlet temperature affected the Py at the 0.01% and 5.0% levels of significance, respectively (Table 3). The linear term of AM was significant at 5.0%. However, the interactions between factors were not significant (Table 3). The surface plot in Fig. 1 shows that increasing temperature resulted in a significant increase in yield. This could be due to an increase in drying rate, thereby increasing the probability that particles are dry when they reach the dryer walls and thus avoiding adhesion to the chamber. Polyvinylpyrrolidone (PVP) is a polymer that increases the solubility of poorly soluble compounds, increasing their bioavailability (Sethia & Squillante, 2004). However, stickiness on the chamber walls during spray drying is due to the presence of PVP in the formulation, as shown in the yield as a function of PC and T, indicating that the decrease of PC promotes an increase in yield (Fig. 2).

The colloidal silicon dioxide is widely used in pharmaceutical formulations generally to improve flowability of powders (Jonat et al., 2004). Couto et al. (2011) dried *Eugenia dysinterica* DC extracts by spray drying using 25% of silicon in formulation obtaining 69.15% of yield. In addition, Araújo et al. (2010) showed that the addition of colloidal silicon dioxide at a ratio of 0.5 to 1.5 in spray drying process increased the yield of microparticles containing curcumin and Gelucire® 44/14. However, Fig. 1 shows that the increase in AM decreased the microparticles yield, which can be explained by the silicon effect on solution viscosity. Preliminary experiments in this work indicated that larger silicon ratios, AM, produced highly viscous suspensions, inadequate for atomization. Taking into account only the significant terms, an equation was fitted by response surface analysis and resulted in a correlation coefficient of $R^2 = 0.9819$ (Eq. (7)).

$$\text{Py}(\%) = 40 - 3.12 \times \left(\frac{\text{AM}-0.5}{0.2}\right) - 9.37 \times \left(\frac{\text{PC}-3}{2}\right) + 8.25 \times \left(\frac{\text{T}-60}{20}\right) - 4.87 \times \left(\frac{\text{T}-60}{20}\right)^2 - 3.12 \times \left(\frac{\text{PC}-3}{2}\right)^2. \quad (7)$$

Table 3
Analysis of variance for process yield (Py).

Factors	Sum of squares	Degree freedom	Mean squares	*p*
T	544.500	1	544.500	0.000136*
T^2	87.750	1	87.750	0.008405*
AM	78.125	1	78.125	0.010607*
AM^2	0.058	1	0.058	0.918227
PC	703.125	1	703.125	0.000073*
PC^2	36.058	1	36.058	0.042837*
T × AM	9.000	1	9.000	0.235387
T × PC	25.000	1	25.000	0.074526
AM × PC	2.250	1	2.250	0.530092
Error	24.750	5	4.950	

* $p<0.05$.

3.3. Microparticle morphology

SEM of the microparticulates prepared at 60 °C, ratio of colloidal silicon dioxide to *C. longa* extract and PVP 0.3 and ratio of PVP to *C. longa* extract of 5.0 showed the particles to be spherical in shape, with low surface roughness and the formation of few agglomerates of microparticles (Fig. 3).

3.4. Total curcuminoid content (TCC)

The TCC for the samples ranged from 17.15 to 19.57 (Table 2). The statistical analysis showed that neither the individual factors nor their

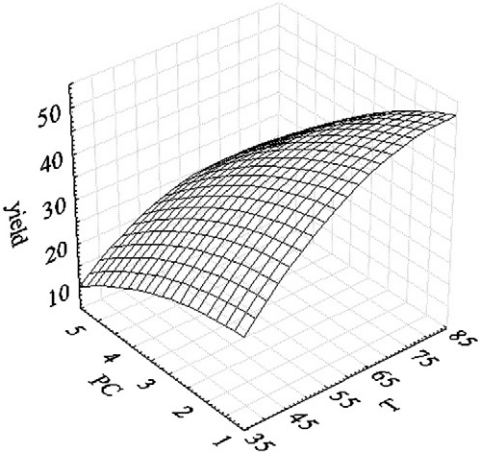

Fig. 2. Yield of microparticles as function of temperature (T) and the ratio of PVP to curcumin (PC).

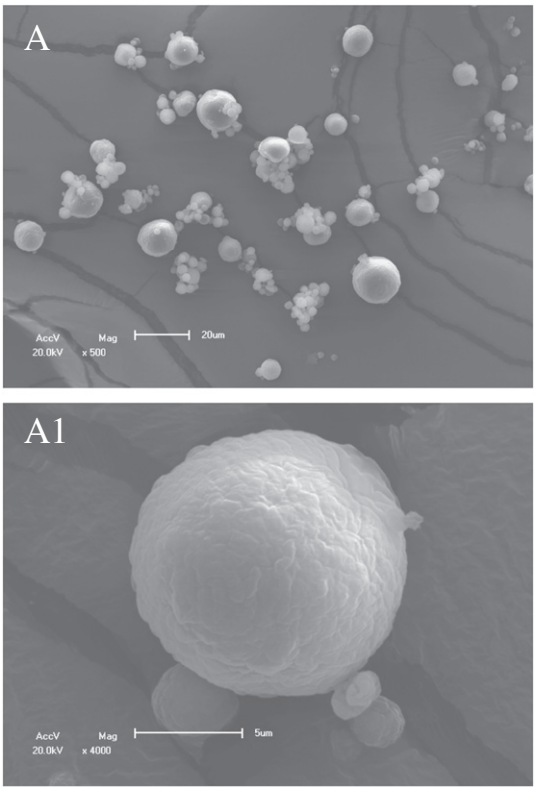

Fig. 3. SEM micrographs of microparticles obtained at 60 °C of *C. longa* extract (A = 500× and A1 = 4000×).

interactions terms affected the TCC significantly (Table 4). However, the squared terms of T and PC were significant at 0.05% and 5.0%, respectively. As confirmed by the ANOVA, there are curvatures in both effects, which are related to the quadratic terms of T and PC. The surface plot in Fig. 4 shows that the highest TCC occurs at intermediate values for both factors. A polynomial equation was fitted to experimental data (Eq. (8)) considering only the factors with effects significant at 5.0%. Eq. (8) resulted in a squared correlation coefficient (R^2) of 0.9470.

$$\text{TCC (mg/g)} = 16.58 + 0.56 \times \left(\frac{\text{PC}-3}{2}\right)^2 - 1.67 \times \left(\frac{\text{T}-60}{20}\right)^2. \quad (8)$$

3.5. Curcumin content (CC)

The concentration of CC for microparticles ranged from 3.24 to 4.25 mg/g, as shown in Table 2. The ANOVA of the CC data is presented

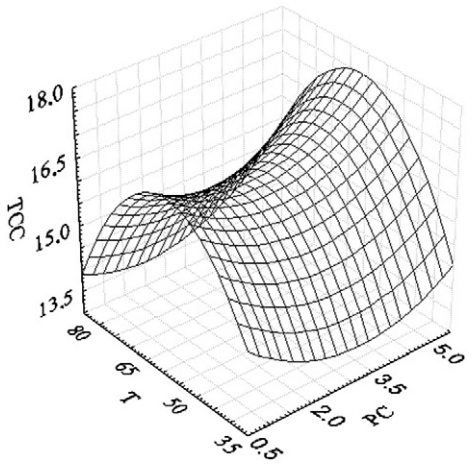

Fig. 4. Total curcuminoid content as function of temperature (T) and the ratio of PVP to curcumin (PC).

in Table 5. The AM and PC influenced the CC at significance levels of 5.0% and 0.5%, respectively. In addition, the interaction term between the AM and PC was significant at 0.5%. The surface plot in Fig. 5 shows that with increasing AM and a decrease in PC, the content of curcumin (CC) decreases. These results suggest that the PVP provides formulation stability by preventing the degradation of curcumin during the production of microparticles by spray drying. This hypothesis is supported by comparing the CC of lyophilized extract (4.06 mg/g) with microparticle spray dried at 60 °C, AM of 0.3 and PC of 1.0 (3.24 mg/g). The response surface analysis resulted in Eq. (9) for the prediction of CC with correlation coefficient $R^2 = 0.9471$.

$$\text{CC (mg/g)} = 3.69 - 0.11 \times \left(\frac{\text{AM}-0.5}{0.2}\right) + 0.18 \times \left(\frac{\text{PC}-3}{2}\right) - 0.35 \times \left(\frac{\text{AM}-0.5}{0.2}\right) \times \left(\frac{\text{PC}-3}{2}\right). \quad (9)$$

3.6. Antioxidant activity (DPPH• test)

Effects of the spray drying conditions on the IC_{50} of the microparticles are shown in Fig. 6. The antioxidant activity, determined using the DPPH• method, resulted in IC_{50} values ranging from 530.1 to 860.3 μg/mL (Table 2). The effects of both the drying proportion of PC and the proportion of AM appear to be nonlinear, and the lowest antioxidant activities are found at intermediate values of PC and AM (Table 6). The linear and interaction terms were not significant, but the quadratic terms of AM and PC were significant at 1.0% and 5.0%, respectively. The response surface analysis allows for

Table 4
Analysis of variance for total curcuminoid content (TCC).

Factors	Sum of squares	Degree freedom	Mean squares	*p*
T	0.0066	1	0.0066	0.847260
T^2	10.2821	1	10.2821	0.000493*
AM	0.2178	1	0.2178	0.296908
AM^2	0.9463	1	0.9463	0.059641
PC	0.4278	1	0.4278	0.163715
PC^2	1.1631	1	1.1631	0.043295*
T × AM	0.2209	1	0.2209	0.293869
T × PC	0.3192	1	0.3192	0.217798
AM × PC	0.4489	1	0.4489	0.155543
Error	0.8036	5	0.1607	

* $p<0.05$.

Table 5
Analysis of variance for curcumin content (CC).

Factors	Sum of squares	Degree freedom	Mean squares	*p*
T	0.03380	1	0.03380	0.133310
T^2	0.00023	1	0.00023	0.887987
AM	0.09461	1	0.09461	0.030227*
AM^2	0.02388	1	0.02388	0.192600
PC	0.24851	1	0.24851	0.004649*
PC^2	0.00908	1	0.00908	0.395947
T × AM	0.03240	1	0.03240	0.139904
T × PC	0.00250	1	0.00250	0.646815
AM × PC	0.49703	1	0.49703	0.001001*
Error	0.05269	5	0.01054	

* $p<0.05$.

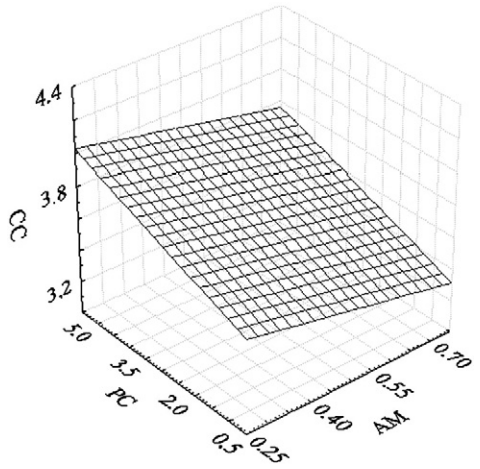

Fig. 5. Curcumin content as function of the ratio of silicon to curcumin–PVP mixture (AM) and the ratio of PVP to curcumin (PC).

the fitting of IC_{50} as a function of the significant factors. The fitted equation is given by Eq. (10), with correlation coefficient $R^2 = 0.905$.

$$IC_{50}(\mu g/mL) = 819.33 - 134.67 \times \left(\frac{AM-0.5}{0.2}\right)^2 - 113.42 \times \left(\frac{PC-3}{2}\right)^2. \quad (10)$$

3.7. Solubility

Based on the results of TCC, CC and IC_{50}, experiments 9, 10, 11 and 14 were chosen for solubility evaluation, as they represent the extreme values of these properties. The microparticles of *C. longa* showed increased solubility for the TCC when compared to the *C. longa* extract (0.22 μg/mL ± 0.03), as shown in Fig. 7. The microparticles from experiment 11 (21.30 μg/mL ± 2.48) enhanced TCC solubility more than the microparticles from experiments 9 (4.94 μg/mL ± 0.41), 10 (4.39 μg/mL ± 0.36) and 14 (5.05 μg/mL ± 0.88), where results were statistically similar. According to Araújo et al. (2010) the increase in colloidal silicon dioxide content in SD decreases the solubility of curcumin in microparticles produced by spray drying. In the present study, it seems that the values of AM for experiments 9, 10 and 14 did not affect the solubility of TCC of the microparticles. In particular, experiment 11 increased the TCC solubility from *C. longa* extract approximately 100-fold. The enhanced solubility is likely due to the high concentration of PVP K30 in these microparticles. PVP has been the most common carrier used for solid dispersions. The high molecular weight (approximately 50,000) of PVP K30 favors the formation of solid solutions, increasing the solubility of poorly soluble drugs (Sharma, Soni, Kumar, & Gupta, 2009). The increase in TCC solubility from *C. longa* extract by PVP may be due to the formation of soluble complexes between the water-soluble polymeric carrier and curcuminoids present in extract, as suggested by Sethia and Squillante (2004) for carbamazepine SD in PVP K30 prepared by solvent evaporation and supercritical fluid. The increased solubility of curcumin and other curcuminoids in microparticulated preparations is an important and promising result. Increased solubility leads to increased coloring properties as well increased bioavailability of these substances (Leuner & Dressman, 2000), creating opportunities for new applications in the food and nutraceutical industries.

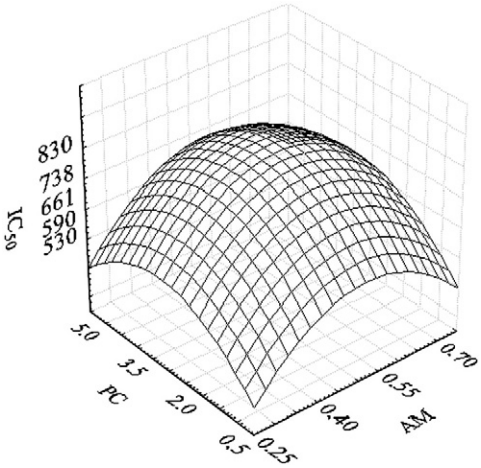

Fig. 6. Antioxidant activity, IC_{50}, as function of the ratio of silicon to curcumin–PVP mixture (AM) and the ratio of PVP to curcumin (PC).

Table 6
Analysis of variance for IC_{50} by DPPH• method.

Factors	Sum of squares	Degree freedom	Mean squares	*p*
T	4005	1	4005	0.298708
T^2	7532	1	7532	0.172760
AM	10440	1	10440	0.120164
AM^2	66960	1	66960	0.005150*
PC	12012	1	12012	0.100953
PC^2	47495	1	47495	0.010406*
T × AM	1	1	1	0.986094
T × PC	6006	1	6006	0.214952
AM × PC	506	1	506	0.697310
Error	14902	5	2980	

* $p < 0.05$.

4. Conclusion

The spray drying technique was successfully used to prepare microparticles of *C. longa* extract using PVP and colloidal silicon dioxide with spherical shape, adequate morphology and curcuminoid content. All variables analyzed (yield, TCC, CC and IC_{50}) indicated that the spray drying process should be controlled using optimized conditions. The solubility of microparticles of *C. longa* improved 100-fold compared to *C. longa* extract. The results confirm the commercial potential of spray drying to obtain microparticles of poorly water-soluble phytochemicals.

Acknowledgments

Financial support from FAPESP (08/02848-9 and 08/07115-0) and CNPq (PQ-2) are gratefully acknowledged.

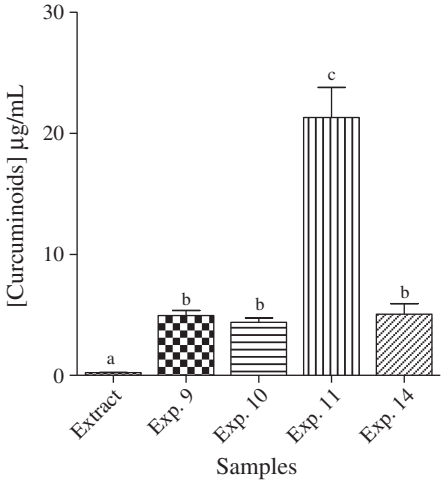

Fig. 7. Solubilities in water of *Curcuma longa* L extract, and microparticles from experiments 9, 10, 11 and 14. Statistical analysis was performed by one-way ANOVA followed by Bonferroni's test of multiple comparisons. Values with same superscript letter are statistically equal at $p < 0.05$ (analysis of variance).

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