Ciência e Tecnologia de Alimentos
ISSN: 0101-2061
revista@sbcta.org.br
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Brasil

de Moraes CARVALHO, Deivis; Pereira TAKEUCHI, Katiuchia; Maia GERALDINE, Robson; de MOURA, Celso José; Lopes TORRES, Maria Célia
Production, solubility and antioxidant activity of curcumin nanosuspension
Ciência e Tecnologia de Alimentos, vol. 35, núm. 1, enero-marzo, 2015, pp. 115-119
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Campinas, Brasil

DOI: http://dx.doi.org/10.1590/1678-457X.6515

Production, solubility and antioxidant activity of curcumin nanosuspension

Deivis de Moraes CARVALHO[1*], Katiuchia Pereira TAKEUCHI[1], Robson Maia GERALDINE[1], Celso José de MOURA[1], Maria Célia Lopes TORRES[1]

Abstract

Curcumin is a powerful bioactive agent and natural antioxidant, but it is practically water-insoluble and has low bioavailability; a possible solution to this obstacle would be formulations of curcumin nanoparticles. Surfactants such as tween 80 can be used to stabilize low-solubility molecules preventing particle aggregation. The objectives of this study were the preparation of a suspension with curcumin nanoparticles in tween 80, the testing of pure curcumin solubility and of a simple mixture of curcumin with tween 80 and nanosuspension in water and ethanol as solvents, and finally the assessment of the antioxidant activity. We prepared the nanosuspension by injecting a curcumin solution in dichloromethane at low flow in water with tween 80 under heating and ultrasound. The analysis of particles size was conducted through dynamic light scattering; the non-degradation of curcumin was verified through thin-layer chromatography. The analyses of antioxidant activity were carried out according to the DPPH method. The method applied to reduce the particles size was efficient. Both the curcumin suspension and nanosuspension in tween 80 increased its solubility. Curcumin and the formulations presented antioxidant activity.

Keywords: curcumin; nanoparticles; solubility; antioxidant.

Practical Application: Increase the use of curcumin as a colorant and antioxidant.

1 Introduction

Curcumin is a yellow-orange compound extracted from curcuma rhizomes, especially Curcuma longa L, obtained through extraction with solvent and extract purification through crystallization. The chemical composition of the commercial product is often the mixture of curcuminoids derived from curcuma in varied proportions. The concentration of the three major curcuminoids of different samples of Curcuma longa L presented an average composition of 5.69% - 2.86% curcumin, 1.47% desmethoxycurcumin and 1.36% bidesmethoxycurcumin (Li et al., 2011). The fields of food and pharmacy have extensively researched curcumin with models in vitro to analyze the curcuminoids antioxidant activity. The studies presented results demonstrating that the desmethoxycurcumin and the bisdesmethoxycurcumin along with curcumin are also satisfactory antioxidants. It could be suggested the use of those three compounds in food systems to increase shelf life (Jayaprakasha et al., 2006).

Despite being a powerful bioactive agent and natural antioxidant, curcumin is practically water-insoluble and has low bioavailability (Modasiya & Patel, 2012); a possible solution to this obstacle would be the development of formulations of curcumin nanoparticles to improve its stability (Mohanty & Sahoo, 2010). The higher water-solubility could be attributed to a larger surface area in contact with the solvent. Both the curcumin and the nanocurcumin have the same chemical structure. The nanocurcumin aqueous dispersion had an antimicrobial effect stronger than the curcumin (Bhawana et al., 2011). Studies have been approaching different nanocurcumin formulations with technological potential to improve its bioavailability to treat diseases such as cancer (Yallapu et al., 2012). Nanotechnology has become multidisciplinary in the field of technology and applied sciences; it involves the study of nanoparticles and the capacity of working with different materials in nanometric scale in order to understand, create, characterize and use materials with new properties derived from their nanostructures (Azeredo, 2009). Several are the methods to obtain nanoparticles such as antisolvent precipitation, low flow injection, evaporation, precipitation and nanosuspensions applied to reduce the curcumin particles size (Kakran et al., 2012). Surfactants can be used to stabilize low-solubility molecules, therefore preventing particle aggregation. The monooleate of ethoxylated sorbitan 80 – also known as polysorbate 80 or tween 80 (INS 433) – is a surfactant and nonionic emulsifier used as dispersing agent by other authors who worked with nanoparticles (Kakkar et al., 2011; Lin et al., 2009; Bihari et al., 2008). As curcumin (INS 100i), its use is allowed in food and pharmaceutical industries (Codex Alimentarius - International Food Standards, 2009, 2013a, 2013b). The objectives of this study were to prepare a suspension with curcumin nanoparticles in tween 80 (nanosuspension), to test the solubility of pure curcumin, of a simple mixture of curcumin with tween 80 and of the nanosuspension in water and ethanol as solvents, and finally to assess the antioxidant activity.

Received 21 Oct., 2014
Accepted 26 Jan., 2015
[1]*Escola de Agronomia, Universidade Federal de Goiás – UFG, Campus Samambaia, Goiânia, GO, Brasil*
*Corresponding author: deivism.carvalho@gmail.com

2 Materials and methods

The analyzed samples were constituted of pure curcumin (PC) (Sigma Aldrich, 65% curcumin, 35% other curcuminoids), simple mixture with 22.2% curcumin in tween 80 (SM) and nanosuspension with 22.2% curcumin in tween 80 (NS).

2.1 Nanosuspension production

To obtain the nanosuspension, we injected a 4 mL solution (0.5% p/v) of curcumin solubilized in dichloromethane P.A (99.5%) in a 100 mL aqueous solution (0.07% p/v) of tween 80 using an infusion pump (Insight Equipamentos LTDA, Ribeirão Preto, Brazil), at a 0,2 mL flow per minute (Bhawana et al., 2011) under heating (65 ± 5 °C) in ultrasound bath (Digital Ultrasonic Cleaner, model D409X, CTA do Brasil, Brazil) with 40 kHz frequency and 400 W power. After fours batches kept under agitation, the 400 mL obtained were concentrated at 40 mL in rotary evaporator (Quimis, model 344B2, Diadema, Brazil) at 60 °C under vacuum (550 mmHg). Subsequently, the mixture was frozen in ultra freezer (Coldlab, model CL374-80V, Piracicaba, Brazil) at – 80 °C for 2 hours and lyophilized (Lyophilization equipment Liobras, model Liotop LP510, São Carlos, Brazil) obtaining the nanosuspension with 22.2% nanocurcumin in tween 80. The nanosuspension was maintained in the dark, without humidity.

2.2 Particles size

We analyzed the curcumin nanoparticles dimension in the suspension by establishing their hydrodynamic diameter through dynamic light scattering using a Zeta sizer (Malvern Instruments, Zeta sizer nano series Nano-s, United Kingdom) with the references of refractive index and viscosity of pure water (Feng et al., 2012); 4.5 mg of the nanosuspension were solubilized in 10 mL deionized water and centrifuged water at 5,500 rpm for 20 minutes.

2.3 Thin-layer chromatography (TLC)

In order to verify whether the curcumin molecules suffered degradation during the nanosuspension processing, we submitted the samples to an analysis of thin-layer chromatography, comparing the simple mixture (SM) of curcumin in tween 80 (or control sample) and the curcumin nanosuspension sample (NS). We used the mobile phase of chloroform-methanol (Modasiya & Patel, 2012; Bhawana et al., 2011) at concentrations 1% and 20% methanol; the stationary phase involved aluminum foil with 0.20 mm silica gel 60 with fluorescent indicator UV254 (Macherey Nagel, Düren, Germany).

2.4 Solubility analysis

The entire procedure was carried out in a room conditioned at 25 °C. The solubility was tested in deionized water and in ethanol P.A. (99.5%). The samples were added in quantities sufficient to reach excess curcumin content in the solvent exceeding the saturation point. We prepared the samples test tubes with screw cap covered with aluminum foil to avoid exposition to the light. After combining the sample and the solvent, we conducted a 2-3-minute homogenization using a vibration homogenizer (Biomixer Vortex, model QL-901, Brazil) subsequently placing the tubes in a rotating homogenizer (Phoenix, model AP32, Araraquara, Brazil) for 24 horas. After the homogenization, the samples were filtered in filter paper (Unifil blue strip C42, 2.0 micrometers porosity) at atmospheric pressure (Modasiya & Patel, 2012). To establish the concentrations of solubilized curcumin, we used the NBR 13624 (Associação Brasileira de Normas Técnicas, 1996) methodology with alterations for the stage of preparing the samples and a standard curve (absorbance/concentration). The readings were conducted through spectrophotometer (Biospectro, model SP-220, Curitiba, Brazil); every solvent without the solute was used as "white". The filtered samples were analyzed in 425 nm wavelength; the concentrations enabled the quantification of the solubilized curcumin mass in each solvent.

2.5 Antioxidant analysis

The analyses were carried out according to methodology by Rufino et al. (2007) by replacing the fruit extracts with solutions of 0.1 mM; 0.2 mM and 0.4 mM curcumin or L-ascorbic acid (AA) (comparison parameter) solubilized in methanol. Three curcumin preparation were analyzed: pure curcumin (PC); simple mixture of curcumin with tween 80 (SM) and curcumin nanosuspension in tween 80 (NS).

2.6 Statistical analysis

We prepared the samples were with 3 repetitions and carried out the analyses in triplicate, followed by analysis of variance and Tukey test ($p \leq 0.05$).

3 Results and discussion

3.1 Reduction of particles size

The analyses of the mean hydrodynamic diameter of the particles resulted in 118.6 nm. Figure 1 illustrated a graph of particles distribution of an analysis using the Zeta sizer, where 92.2% of the particles (peak 1) presented mean diameter of 102.1 nm.

Also according to description by Kaewnopparat et al. (2009), the nanoparticle pure curcumin demonstrate a slow and low rate of dissolution in water due to its hydrophobicity that causes the flowing powder in the surface of the dissolution area, reducing the contact area with the solvent. When slowly injected in dichloromethane in the aqueous area under heating (65 ± 5 °C) and ultrasound, the solubilized curcumin spread simultaneously with the dichloromethane (39.8 °C boiling point) rapidly volatilization. Bhawana et al. (2011) used injections with lower curcumin solutions and batches to report that they achieved satisfactory stability of curcumin nanoparticles without adding surfactants. We used batches with higher concentrations at the initial phase of preparation. To avoid the coalescence of curcumin nanoparticles, we used tween 80 in the distilled water that was injected with curcumin solution. After the material concentration, we obtained the nanosuspension with curcumin nanoparticles in tween 80.

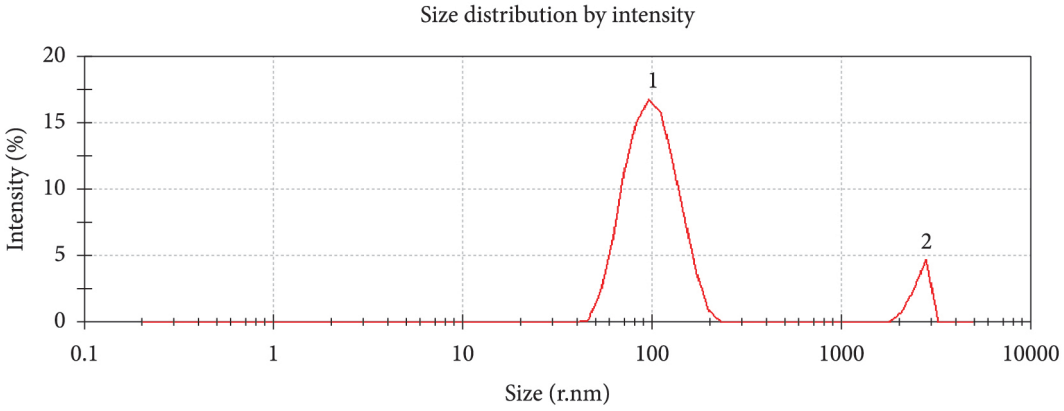

Figure 1. Distribution of curcumin particles size. Graph generated through Zeta sizer (Malvern Instruments, Zeta sizer nano series Nano-s, United Kingdom).

During the concentration, the aggregation of surfactant molecules probably forms micelles with a critical micelle concentration that could provide the required influence to stabilize the curcumin molecules (Yallapu et al., 2012). By removing the water, the particles would be suspended in the tween 80; the nanosuspension could be solubilized again in the desired solvent when required.

3.2 Solubility assessment

We tested the solubility of pure curcumin (PC), of a simple mixture with 22.2% curcumin in tween 80 (SM) and a nanosuspension containing 22.2% curcumin in tween 80 (NS). Significant differences were found with results presented in Table 1.

The water-solubility of curcumin in the SM was 216 times higher than the PC. The solubility of curcumin in NS was 1,936 times higher than the PC and 9 times higher than the SM. These results indicate that the use of suspensions with tween 80 have evident potential as dispersing agent of curcumin in aqueous formulations both in the form of nanosuspensions (complex process - more efficient but expensive) and in the form of simple mixtures (simple process - less efficient and cheaper). Figure 2 brings the image of the saturated solutions of SM and NS in water; the different coloring is due to the different concentrations of solubilized curcumin. Both solutions were prepared with pH 5.8.

The PC solubility in ethanol of is much higher than in water, even in the form of NS in water. In ethanol, no significant difference was indicated for the PC to the SM since the tween 80 had no surfactant action in the alcoholic medium. In the form of NS, the ethanol-solubility was more than doubled demonstrating its potential to reduce the particles size and improve the solubility of curcumin also in ethanol.

The decreased particles size increased the surface of contact with the solvent and consequently the solubility. However, when diluted in water in the absence of a proper stabilizer, the nanoparticle surface becomes prone to clustering, while a good stabilizer could spread the particle in the interface with water to prevent aggregation (Gao et al., 2011). Therefore, the tween 80 served to prevent the curcumin from re-aggregating in the aqueous solutions.

Table 1. Solubility of curcumin preparations in water and in ethanol.

Solvent	Preparation	Solubilized curcumin (mg.L–1)
Distilled water	PC	1.34 ± 0.02 a
Distilled water	SM	289.41 ± 5.03 b
Distilled water	NS	2,594.39 ± 90.09 c
Ethanol 99.5%	PC	8,895.89 ± 737.26 d
Ethanol 99.5%	SM	8,481.15 ± 555.70 d
Ethanol 99.5%	NS	18,634.10 ± 1,339.85 e

PC – Pure Curcumin, SM – Simple Mixture, NS – Nanosuspension. Different letters (a, b, c, d, e) represent significant difference ($p \leq 0.05$).

3.3 Thin-layer chromatography (TLC)

We carried out analyses of Thin-layer chromatography (TLC) of the NS using the SM as control to verify whether there had been any alterations in the curcumin processed (solubilized in dichloromethane, ultrasound, concentration under heating and lyophilization) to obtain the NS. Table 2 presents all the results.

Equal mobile phases presented equal Retention Factor (FR) for the samples indicating equal substances. The results point out that the process of producing the nanosuspension did not degrade the curcumin.

3.4 Antioxidant activity

The DPPH method (2.2-diphenyl-1-picryl hydrazyl) is based on the capture of the DPPH free radical by antioxidants, producing a decrease in absorbance at 515 nm wavelength. It is a free radical that can be solubilized directly in methanol (Brand-Wiliams, et al., 1995). The DPPH is stable at room temperature and produces a violet solution in organic solvents such as methanol, ethanol, etc; it is reduced in the presence of curcumin, which decreases its coloring. The use of DPPH provides an easy, quick path to assess the antioxidant properties of curcumin (Kakran et al., 2012). Table 3 indicates the results of the analyses of antioxidant activity carried out according to the

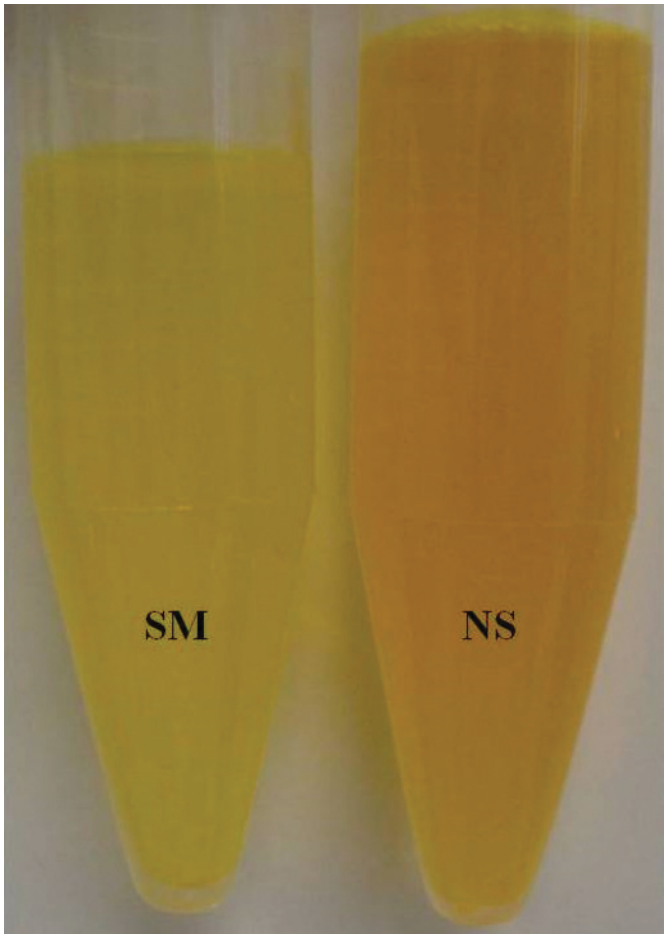

Figure 2. Saturated solutions of simple mixture (SM) and nanosuspension (NS) in distilled water.

Table 2. Thin-layer chromatography of curcumin preparations (TLC).

Mobile phase mixture	RF control (SM)	RF nanosuspension (NS)
1% methanol in chloroform	0.24 ± 0.01 a	0.24 ± 0.01 a
20% methanol in chloroform	0.70 ± 0.02 b	0.70 ± 0.01 b

RF – Retention Factor of curcumin, SM – Simple mixture (control), NS – Nanosuspension. Different letters (a, b) represent significant difference ($p \leq 0.05$)

Table 3. Antioxidant activity (EC50 expressed in g of sample / g of DPPH).

PC	SM	NS	AA
14.09 ± 0.60 a	18.50 ± 1.09 b	19.83 ± 1.48 b	6.99 ± 0.19 c

PC – Pure curcumin, SM – Simple mixture, NS – Nanosuspension, AA – L-ascorbic acid. Different letters (a, b, c) represent significant difference ($p \leq 0.05$).

DPPH method, which requires the solubilization of the DPPH and the analyzed samples in methanol. The result (EC50) was expressed in grams of consumed sample per gram of DPPH free radical. The ascorbic acid (AA), a common antioxidant in foods, was used as reference and presented higher antioxidant activity than the curcumin.

No significant difference was indicated between the antioxidant activity of the SM and the NS, which could be explained by the high solubility of curcumin in methanol even as macro particle. As every curcumin of both samples were totally solubilized, the kinetics of the molecules reaction in the solvent and the results were equal. Certainly, if instead of having used samples with equal curcumin concentrations, we would have used saturated samples, the NS would have been lower than the SM since the concentration of the solubilized NS would have been lower.

Kakran et al. (2012) worked without adding surfactants and verified that the antioxidant activity studied through the elimination of DPPH free radicals was higher for the nanoparticles than for the original curcumin; the authors attribute it to the higher solubility of nanoparticles. The curcumin has probably proved antioxidant effect especially due to its phenolic hydroxyl groups. Our study revealed significant difference, and since the PC had been completely solubilized in methanol, in presented better antioxidant activity than the SM and NS formulations. It probably occurred because the molecules of the tween 80 present in the area increased the viscosity therefore decreasing the mobility and also competing for the interactions with molecules of curcumin, methanol and DPPH, which reduced the shocks between curcumin and DPPH molecules.

We suggest that both the SM and the NS formulations can be used in water/ethanol-based food or medicines as coloring, antioxidant or active principle.

4 Conclusions

The method applied to reduce the particles size proved efficient. The process of preparing the nanosuspension did not degrade the curcumin molecules. The nanosuspension proved more soluble than the simple mixture. The simple mixture of curcumin in tween 80 increased its water-solubility in contrast to ethanol, while the nanosuspension increased its water-solubility even more, and also its ethanol-solubility. The pure curcumin and the studied formulations presented satisfactory antioxidant activity, although lower than the ascorbic acid. The reduction in the particles size with tween 80 did not improve the curcumin antioxidant activity.

Acknowledgements

Financial support: MCT/SEBRAE/FINEP, Work Plan nº 04/2007 – cooperation ICT's/MPE's, in APL's – nº 01/2007: Potencializando o açafrão de Mara Rosa - GO.

References

Associação Brasileira de Normas Técnicas – ABNT. (1996). *NBR 13624. Cúrcuma – Determinação do teor de curcumina – Método de ensaio*. Rio de Janeiro: ABNT.

Azeredo, H. M. C. (2009). Nanocomposites for food packaging applications. *Food Research International*, *42*(9), 1240-1253. http://dx.doi.org/10.1016/j.foodres.2009.03.019.

Bhawana, Basniwal, R. K., Buttar, H. S., Jain, V. K., & Jain, N. (2011). Curcumin nanoparticles: preparation, characterization, and antimicrobial study. *Journal of Agricultural and Food Chemistry*, *59*(5), 2056-2061. http://dx.doi.org/10.1021/jf104402t. PMid:21322563

Bihari, P., Vippola, M., Schultes, S., Praetner, M., Khandoga, A. G., Reichel, C. A., Coester, C., Tuomi, T., Rehberg, M., & Krombach, F. (2008). Optimized dispersion of nanoparticles for biological in vitro and in vivo studies. *Particle and Fibre Toxicology*, *5*(1), 14. http://dx.doi.org/10.1186/1743-8977-5-14. PMid:18990217

Brand-Wiliams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25-30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5.

Codex Alimentarius – International Food Standards. (2009). *CODEX STAN 256-2007: Standard for fat spreads and blended spreads*. Rome: Codex Alimentarius.

Codex Alimentarius - International Food Standards. (2013a). *CAC/GL 36-1989: Class names and the international numbering system for food additives*. Rome: Codex Alimentarius.

Codex Alimentarius – International Food Standards. (2013b). *CAC/MISC 6-2013: List of codex specifications for food additives*. Rome: Codex Alimentarius.

Feng, R., Song, Z., & Zhai, G. (2012). Preparation and in vivo pharmacokinetics of curcumin-loaded PCL-PEG-PCL triblock copolymeric nanoparticles. *International Journal of Nanomedicine*, *7*, 4089-4098. http://dx.doi.org/10.2147/IJN.S33607. PMid:22888245

Gao, Y., Li, Z., Sun, M., Guo, C., Yu, A., Xi, Y., Cui, J., Lou, H., & Zhai, G. (2011). Preparation and characterization of intravenously injectable curcumin nanosuspension. *Drug Delivery*, *18*(2), 131-142. http://dx.doi.org/10.3109/10717544.2010.520353. PMid:20939679

Jayaprakasha, G. K., Jaganmohan Rao, L., & Sakariah, K. K. (2006). Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin. *Food Chemistry*, *98*(4), 720-724. http://dx.doi.org/10.1016/j.foodchem.2005.06.037.

Kaewnopparat, N., Kaewnopparat, S., Jangwang, A., Maneenaun, D., Chuchome, T., & Panichayupakaranant, P. (2009). Increased solubility, dissolution and physicochemical studies of curcumin-polyvinylpyrrolidone K-30 solid dispersions. *Engineering and Technology*, *31*, 225-230.

Kakkar, V., Singh, S., Singla, D., & Kaur, I. P. (2011). Exploring solid lipid nanoparticles to enhance the oral bioavailability of curcumin. *Molecular Nutrition & Food Research*, *55*(3), 495-503. http://dx.doi.org/10.1002/mnfr.201000310. PMid:20938993

Kakran, M., Sahoo, N. G., Tan, I., & Li, L. (2012). Preparation of nanoparticles of poorly water-soluble antioxidant curcumin by antisolvent precipitation methods. *Journal of Nanoparticle Research, Arlington*, *14*, 757. http://dx.doi.org/10.1007/s11051-012-0757-0.

Li, S., Yuan, W., Deng, G., Wang, P., Yang, P., & Aggarwal, B. B. (2011). Chemical Composition and Product Quality Control of Turmeric (Curcuma longa L.). *Pharmaceutical Crops*, *5*(1), 28-54. http://dx.doi.org/10.2174/2210290601102010028.

Lin, C. C., Lin, H. Y., Chen, H. C., Yu, M. W., & Lee, M. H. (2009). Stability and characterization of phospholipid-based curcumin-encapsulated microemulsions. *Food Chemistry*, *116*(4), 923-928. http://dx.doi.org/10.1016/j.foodchem.2009.03.052.

Modasiya, M. K., & Patel, V. M. (2012). Studies on solubility of curcumin. *International Journal of Pharmacy & Life Sciences*, *3*(3), 1490-1497.

Mohanty, C., & Sahoo, S. K. (2010). The in vitro stability and in vivo pharmacokinetics of curcumin prepared as an aqueous nanoparticulate formulation. *Biomaterials*, *31*(25), 6597-6611. http://dx.doi.org/10.1016/j.biomaterials.2010.04.062. PMid:20553984

Rufino, M. S. M., Alves, R. E., Brito, E. S., Morais, S. M., Sampaio, C. G., Pérez-Jiménez, J., & Saura-Calixto, F. D. (2007). Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. *Comunicado Técnico*, *127*, 1-4.

Yallapu, M. M., Jaggi, M., & Chauhan, S. C. (2012). Curcumin nanoformulations: a future nanomedicine for cancer. *Drug Discovery Today*, *17*(1-2), 71-80. http://dx.doi.org/10.1016/j.drudis.2011.09.009. PMid:21959306