Comparison of Yield, Composition, and Antioxidant Activity of Turmeric (*Curcuma longa* L.) Extracts Obtained Using Various Techniques

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Turmeric extracts were obtained from two lots of raw material (M and S) using various techniques: hydrodistillation, low pressure solvent extraction, Soxhlet, and supercritical extraction using carbon dioxide and cosolvents. The solvents and cosolvents tested were ethanol, isopropyl alcohol, and their mixture in equal proportions. The composition of the extracts was determined by gas chromatography−flame ionization detection (GC-FID) and UV. The largest yield (27%, weight) was obtained in the Soxhlet extraction (turmeric (S), ethanol = 1:100); the lowest yield was detected in the hydrodistillation process (2.1%). For the supercritical extraction, the best cosolvent was a mixture of ethanol and isopropyl alcohol. Sixty percent of the light fraction of the extracts consisted of ar-turmerone, (*Z*)-γ-atlantone, and (*E*)-γ-atlantone, except for the Soxhlet extracts (1:100, ethanol), for which only ar-turmeronol and (*Z*)-α-atlantone were detected. The maximum amount of curcuminoids (8.43%) was obtained using Soxhlet extraction (ethanol/isopropyl alcohol). The Soxhlet and low pressure extract exhibited the strongest antioxidant activities.

KEYWORDS: ***Curcuma longa* L.; antioxidant and anticancer activities; cosolvents; hydrodistillation; solvent extraction; supercritical extraction; turmeric**

INTRODUCTION

The substances extracted from turmeric (*Curcuma longa* L.), paprika (*Capsicum annuum*), sassafras (*Sassafras albidum*), and other mineral dyes have been used for hundreds of years to impart color to foods and for medicinal preparations and related products. In the middle of the nineteenth century, organic dyes were developed, creating a large variety of coloring additives of economical importance, but it was the natural dyes or natural pigments that revolutionized the market (*1*).

Turmeric (*C. longa* L.) extract is an oleoresin consisting of a light (volatile oil) fraction and a heavy fraction of yellowish-brown color. The compounds responsible for the yellow color are the curcumin (1,7-bis(4-hydroxy-3-methoxyfenil)-1,6-heptadiene-3,5-dione) and two curcuminoids, demethoxy-curcumin and bis-demethoxy-curcumin (*2*−*5*). The amount of oleoresin in the rhizomes varies from 3 to 6% (*6*), and it is predominantly formed of sesquiterpenic ketones (*7*) and 2−8% of curcuminoids (*8*). Curcuminoids have shown antimutagenic activities in different animal and cell cultures. One of the biochemical mechanisms attributed to the anticarcinogenic activity of curcumin is related to its carbonyl group (*9*).

Huang et al. (1991), cited by Araújo and Léon (*10*), showed that curcumin inhibited the epidermal metabolism of the arachnoidic acid via lipoxigenase and cycloxigenase. This demonstrates its anticancer activity (AC), since the inhibition of these enzymes was dependent on the curcumin concentration (5−10 μM). Gomes et al. (*11*) studied the antileishmanial activity of the curcuminoids; the curcumin was more effective than the reference compound, pentamidine isetionate, against *Leishmania amazonensis*. Curcumin has proven to be more potent against *Leishmania major* than pentamidine: 100% of cellular death was observed at 27 μM curcumin (*12*). Mazumber et al. (1995), cited by Araújo and Léon (*10*), demonstrated that curcumin has antiviral activity: the HIV-1 integrase was inhibited by curcumin (IC_{50} = 40 μM).

The extraction of turmeric volatile oil by supercritical fluid extraction (SFE) was studied by Began et al. (*13*); these authors used the surface response methodology to determine the best operating conditions with respect to the total yield. A temperature of 35 °C, a pressure of 225 bar, and a solvent flow rate of 4.72×10^{-5} kg/s were found to be optimal. They also observed that an increase in the temperature (35−55 °C) decreased the total yield at a constant solvent flow rate and that an increase

in solvent flow rate, at constant temperature and pressure, increased the total yield; the turmeric extract composition was not affected by any of the process variables, in the range of conditions tested. Mendéz et al. (*14*) studied the use of ethanol to increase the amount of curcuminoids in the SFE extracts. Ethanol was added to turmeric before the SFE process. At 45 °C and 300 bar, the amount of curcuminoids solubilized was found to be significantly affected by the presence of ethanol.

The use of cosolvents to increase the extraction of a specific group of compounds has been reported in the literature. Chang et al. (*15*) used water and ethanol as cosolvents for the extraction of green tea volatile oil; the use of 95% ethanol as a cosolvent increased the yield four times, with respect to the use of water. Wang et al. (*16*) studied SFE from ginseng roots and proved that the use of cosolvent (6%, molar of ethanol) increased the yield.

Rónyai et al. (*18*) studied the extractions of corn germ with a carbon dioxide−ethyl alcohol mixture at 300 bar and 42 °C, varying the alcohol content from 0 to 10% in the solvent mixture. The alcohol content in the solvent had a strong influence on the rate of the extraction, and 10% of ethyl alcohol by weight was found to be the optimum; the data also showed that increasing the amount of alcohol in the fluid decreased the extraction time and the consumption of CO_2. The solubilities of the corn germ colorants in the alcohol plus CO_2 mixtures are different; using CO_2 + 2.5%, alcohol yielded straw yellow-colored oils, while CO_2 + 10% alcohol solvents produced orange-yellow extracts. Guan et al. (*20*) studied the solubility of stearic acid in CO_2 using the cosolvent mixture (1:1) of ethanol plus acetonitrile. Badalyan (*17*) studied the extraction of ginger oleoresin using ethanol as cosolvent; under subcritical conditions, an addition of 2% of ethanol increased the yield to approximately 10%. Nonetheless, at supercritical conditions, probably due to the retrograde phenomena, the yield at 35 °C was found to be much smaller than that at 25 °C. Zancan et al. (*19*) have determined that the addition of ethanol or isopropyl alcohol significantly affected the content of gingerols and shogaols in ginger SFE extracts.

The solubility of a solute (or a mixture of solute) in a supercritical solvent can be modified by the addition of a cosolvent, due to the strong interactions between the solute and the cosolvent and/or due to the variation of the density of the fluid phase, caused by the addition of the cosolvent. The solubility is found to be dependent on the properties and concentrations of the components of mixture, as well as on the system's temperature and pressure. Molecular associations become more complex when a cosolvent mixture (ethanol + isopropyl alcohol) is added to the system, i.e., the cosolvents can associate with the solvent or with the solute (that is, a mixture in the case of oleoresins) and can self-associate in some cases. The enhancement of the solubility is mainly caused by the formation of clusters of solvent−solute, cosolvent−solute, and solvent−cosolvent−solute (*20*).

The objectives of this work were to compare the extraction yields, chemical composition, and antioxidant activities of turmeric extracts, obtained by SFE, by low pressure solvent extraction (LPSE), by Soxhlet extraction (Soxhlet), and by hydrodistillation (HD). Because curcuminoids are only slightly soluble in carbon dioxide (*13*, *14*), to increase their content in the SFE extracts, it is mandatory to add cosolvent. The use of cosolvent requires its removal from the extracts, thus adding another step to the entire process. Nevertheless, the amounts of cosolvent required are generally very small, as compared to the amounts used in any LPSE process. In a standard low pressure extraction process, for instance, the ratio of solid to solvent easily reaches 1:10. For the SFE process, however, a considerably high amount of cosolvent, like ∼16%, represents a solid to solvent ratio of only ∼1:2. Because the intended use of the turmeric extracts is for the formulation of foods, only ethanol (EtOH) and isopropyl alcohol (IsoC3) were used because they are acceptable as an extraction solvent for food.

MATERIAL AND METHODS

Raw Material Characterization. Two lots of turmeric rhizomes were used. The first one (M) was obtained from the Experimental Unit of EPAMIG (Maria da Fé, Minas Gerais, Brazil), and the second (S) was obtained from Fazenda Experimental Lageado of Unesp (Botucatu, SP, Brazil). Turmeric M and S were cultivated without any specific fertilization; turmeric M was grown in a consortium with peach plantation. The rhizomes were manually collected, cleaned with water, and separated from the central stem before slicing (M) or triturating (S). Turmeric M was dried in a oven at 30 °C for 24 h. Turmeric S was dried using a continuous flow dryer assembled at the College of Agricultural Engineering/UNICAMP. The dryer capacity was 2 kg of humid ginger, the drying temperature was 35 °C, and the drying time was 8 h. The raw material moisture was determined using the Jacobs method (*21*); the contents of starch, protein, and total lipid soluble substances were determined by AOAC methods 32.2.05, 32.1.22, and 32.1.25 (*22*); ash and fibers were determined by AOAC methods 4.1.10 and 4.6.01 (*23*); the reducing sugars content was determined by the Somogyi−Nelson method (*24*). The sliced or triturated rhizomes were dried at 30 °C. The dried materials were accommodated in plastic bags and kept in a domestic freezer (Brastemp, model 7501, São Paulo, Brazil) at −5 °C avoiding any contact with light. Before the assays, the dried rhizomes were triturated using a knife mill (Stein Laboratory Mill, model L2, Germany) in portions of approximately 30 g for 10 s. The size distribution of the comminuted turmeric particles was determined using a standard testing sieve (series Tyler, Abrosinox, Caieiras, Brazil) under mechanical agitation (Produtest, model 3580, Santo Amaro, Brazil). Mesh sizes 24, 32, and 48 were selected for the assays. The particles' true density was determined by helium pycnometry (Micrometrics, model Multivolume pycnometer 1305) in the Analytical Chemistry Facilities of the Institute of Chemistry, IQ/UNICAMP. The apparent bed density was calculated using the mass of feed and the volume of the extractor's cell. The total porosity (bed + particles) was determined using the particles' true density and the bed apparent density. The mean particle diameter was estimated using the procedure recommended by ASAE (*25*).

Extraction Setup and Procedures. *Traditional Processes: HD, LPSE, and Soxhlet Extraction.* The HD extract (volatile oil) was obtained using ∼0.050 kg of dried turmeric; the process took 3 h and 30 min, and the assay was duplicated. The LPSE extracts were obtained using 0.001 kg of turmeric with a refrigerated shaker (Incubadora Refrigerada Orbital, model MA 420, Marconi, Piracicaba, Brazil) at 30 °C and 168 rpm for 6 h. Ethanol (PA, Merck, lots K30916283 231 and K30655783 222) and isopropyl alcohol (PA, Merck, lot K30929034 229) were used, and two levels of solid to solvent ratios were employed, 1:10 and 1:100. The Soxhlet process was done using 0.005 kg of turmeric, ethanol (PA, Merck, lots K30916283 231 and K30655783 222), and isopropyl alcohol (PA, Merck, lot K 28514034 049); the system was kept under reflux for 2 h and 30 min. The yields were calculated as mass of volatile oil divided by mass of dry turmeric.

SFE Procedure. The experimental runs were conducted using a fixed bed SFE unit containing an extraction cell of approximately 221×10^{-6} m^3 (length of 37.5×10^{-2} m and inside diameter of 2.74×10^{-2} m) and maximum allowable pressure of 400 bar, as described by Pasquel et al. (*26*). The fixed bed was formed inside the extraction cell with turmeric particles of mesh sizes 24, 32, and 48; equal amounts for each particle size were used. To obtain a homogeneous fixed bed with constant porosity of 0.60 ± 0.05, 0.126 ± 0.001 kg of turmeric particles was required. To avoid bed channeling, the particles were fed into the extraction cell in portions of ∼1 g and accommodated with the help of a cylindrical rod. The extraction cell containing the raw

material was adapted into the SFE unit. The experimental procedure has been described by Zancan et al. (*19*). The thermostatic bath controlling the CO_2 inlet flow to the pump was set at −10 °C. After reaching thermal equilibrium, the system was slowly pressurized by opening the valve at the extractor's inlet and allowing CO_2 + cosolvent to flow across the extraction cell. Once the system reached and stabilized at the operating pressure, the valves from the extractor's outlet were opened and the extraction process began. The solvent flow continued for 6 h; the system depressurization required another 50−90 min. Samples were collected every 25 min, and the runs were terminated after 6 h. The cosolvent present in the extracts was eliminated using a vacuum oven (Napco, model 5831, 25 in Hg, Winchester, VA) for 24 h. Afterward, the extracts were kept in a dissector coupled to a vacuum pump (Tecnal, model TE-058, Piracicaba, Brazil) for an additional period of 16 or 40 h. The search for the best cosolvent was done using a 3 × 2 factorial design with replication at a predetermined solvent flow rate of $(4.2 \pm 0.2) \times 10^{-5}$ kg/s, temperature of 30 °C, selected based on the results of Began et al. (*13*), and a fixed amount of cosolvent of (6.4 ± 0.6)% (wt). Ethanol (PA, Merck, 99.8% purity), isopropyl alcohol (PA, EM Science, 99.8% purity), and their 50:50 mixture were used. Pressures of 200 and 300 bar were tested. Carbon dioxide 99.5% (food grade, White Martins Gases Industriais) was used. The overall extraction curves (OECs) were obtained using the cumulated mass of solute and corresponding time intervals. The samples collected during the decompression step were discharged to build the OECs, while the extract retained in the adsorbent column was considered in the total yield. To evaluate the effects of the solvent flow rate and of the amount of cosolvent, additional experiments were performed at 30 °C, 300 bar, and solvent flow rates of 0.8×10^{-5} to 4.0×10^{-5} kg/s, and the amount of cosolvent (ethanol plus isopropyl alcohol, 1:1 v/v) was varied from 6.7 to 16.1% (wt).

Characterization of the Turmeric Extracts. *Chromatographic Analysis.* The turmeric extracts (volatile oil or extract's light fraction) were analyzed in a gas chromatography−flame ionization (GC-FID) system (Shimadzu, GC-17A, Kyoto, Japan) equipped with a capillary column DB-5 (30 m × 0.25 mm × 0.25 μm, J&W Scientific, Folsom, CA). The carrier gas was helium (1.7 mL/min, 99.9% pure, White Martins Gases Industriais), and 1 μL of sample was injected. The sample split ratio was 1/35. The column was heated at 50 °C for 5 min, programmed at 5 °C /min to 280 °C, and held at 280 °C for 5 min. The temperatures of the injector and detector were 230 and 250 °C, respectively. The identification and quantification of the chemical constituents were based on the comparison of the substances' retention times with the data for the same system, previously obtained in our laboratory (*27*).

Thin-Layer Chromatography (TLC). The turmeric extracts were fractionated by TLC. The SFE extracts displacement was compared to that of the HD and Soxhlet extracts. The TLC was performed using silica plates (GF254, Merck 20 cm × 20 cm, 1 mm height, lot 940367895) and a mixture 80:20 of hexane (PA, Merck, lot K24252074732) and ethyl acetate (PA, Merck, lot K25488323837). The plates were revealed with a solution of anisaldehyde.

The quantification of the curcuminoids was done using a spectrophotometer (Hitachi, model 3000, UV−visible, Tokyo, Japan); the absorbance was read at 427 nm. The calibration curve was established using curcuminoids of 90% purity (ITAL, Campinas, Brazil) and the following procedure: 0.0053 g of curcuminoids was diluted to 100 mL with ethanol (99.8% PA, Merck, lot K28659183 104). Aliquots of 2.5 mL (0.25 mL apart) were diluted to 25 mL with ethanol.

Functional Properties of the Turmeric Extracts. To assess the effect of the process on the functional properties of the turmeric extracts, the antioxidant activity (AA) and AC of the extracts were determined. (i) The AA was evaluated using the coupled oxidation of linolenic acid and β-carotene. The methodology of Hammerschmidt and Pratt (*28*) was used with the required modifications for the SFE extracts (*27*). The reaction substrate was prepared using 10 mg of β-carotene (99%, Acros, lot B0070834, Pittsburgh, PA), 10 mL of chloroform (99.0% PA, Ecibra, lot 13017, Santo Amaro, Brazil), 60 mg of linolenic acid (99%, Sigma Chemical Co., lot U-59A-D4-G, St. Louis, MO), and 200 mg of Tween 80 (Synth, PA, Diadema, Brazil). The oxidation reaction was conducted using the following procedure: to 1 mL of substrate was added 2 mL of bidistilled water and 0.05 mL of extract diluted in ethanol (99.8% PA, Merck, lot 1216046030, Rio de Janeiro, Brazil) (0.02 g of extract in 1 mL of ethanol). The mixture was set into a water bath (Tecnal, model TE 159) at 40 °C, and the reaction product was monitored using a spectrophotometer (Hitachi, U-3010) at 0, 1, 2, and 3 h of reaction, by taking absorbance readings at 470 nm. (ii) Briefly, the AC was determined as follows (*27*): Experiments were performed using the following human cancer cell lines: K562 (leukemia), MCF7 (breast), NCIADR (breast expressing the multidrug resistance phenotype), NCI460 (lung), UACC62 (melanoma), OVCAR (ovary), HT29 (colon), PCO3 (prostate), and 786 (kidney). The National Cancer Institute, Frederick, MD (NCI), kindly donated these cell lines, and stock cultures were kept in liquid nitrogen. Cells were cultured in 25 mL flasks (Nunc Brand Products, Roskilde, Denmark) containing 5 mL of RPMI 1640 (Gibco BRL, Life Technologies, São Paulo, Brazil) with 5% fetal bovine serum (Gibco BRL, Life Technologies). The sulforodamine B (SRB) assay was performed according to the method of Skehan (*29*). The cells were fixed by means of protein precipitation with 50% trichloroacetic acid (TCA) (Sigma Chemical Co.) at 4 °C (50 μL/well, final concentration = 10%) for 1 h. The supernatant was then discarded, and the plates were washed five times with tap water. The cells were stained for 30 min with 0.4% of the SRB (Sigma Chemical Co.) dissolved in 1% acetic acid (50 μL/well) (Sigma Chemical Co.) and subsequently washed four times with 1% acetic acid to remove unbound stain. The plates were air-dried, and bound protein stain was solubilized with 150 μL of 10 mM Trizma buffer (Sigma Chemical Co.). The optical density was read on an automated spectrophotometer plate reader at 540 nm. The assays were performed in triplicate. For cells growing in suspension (e.g., leukemia), the same method was employed, but the TCA concentration was 80%, to fix the cells to the bottom.

Table 1. Composition of Dehydrated Turmerics[a] Used in the Present Work: M (Maria da Fé, MG), S (Botucatu, SP); R-S Is the Turmeric Bagasse of Raw Material S

wt %	M	S	R-S
ashes	8.50 ± 0.03	5.915 ± 0.003	7.35 ± 0.03
fibers	3 ± 1	1.6 ± 0.4	2.0 ± 0.3
lipid soluble matter	5.1 ± 0.2	3.4 ± 0.5	0.23 ± 0.05
starch	19 ± 4	34 ± 1	41 ± 3
protein	10.74 ± 0.03	12.2 ± 0.2	14.65 ± 0.05
reducing sugars	7.008 ± 0.003	3.21 ± 0.01	3.20 ± 0.01
moisture (dehydrated turmeric)	8.00 ± 0.02	9.30 ± 0.01	
oleoresin, volatile oil	7.3	7.3	
oleoresin, heavy fraction	1.4	1.4	
not analyzed	30	15	

[a] The moisture contents of turmeric M and S in natura were 85.2 and 66.7% (wt, wet basis), respectively.

Calculation Procedure. Using the experimental data, the OECs were fitted to a spline using two straight lines. The first line was identified with the constant extraction rate period (CER). From the spline, as described by Rodrigues et al. (*30*), the duration of the CER period (t_{CER}) was determined as the time corresponding to the interception of the two straight lines. The slope of the first line was identified as the extraction rate for the CER period (M_{CER}). Using t_{CER} and M_{CER}, the mass ratio of solute (or oleoresin) in the supercritical fluid phase (Y_{CER}) at the extractor's outlet and the yield corresponding to the CER period (R_{CER}) were calculated. The spline fitting was done using the procedures PROC REG and PROC NLIN of SAS 6.12 (*31*). The analysis of variance (ANOVA) was also done with SAS 6.12.

RESULTS AND DISCUSSIONS

Table 1 shows the compositions of the two lots of turmeric used in this work; the compositions of the turmeric bagasses obtained in the SFE are also in the table. Despite being cultivated under different edaphoclimate conditions, the turmeric compositions were similar, except for the content of starch and moisture.

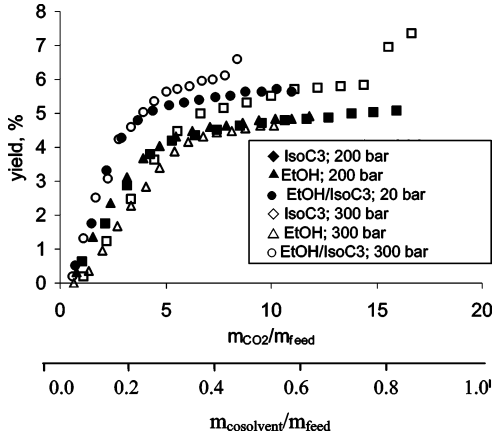

Figure 1. OECs for SFE performed at 30 °C and 6.4 ± 0.6% (v/v) of cosolvent.

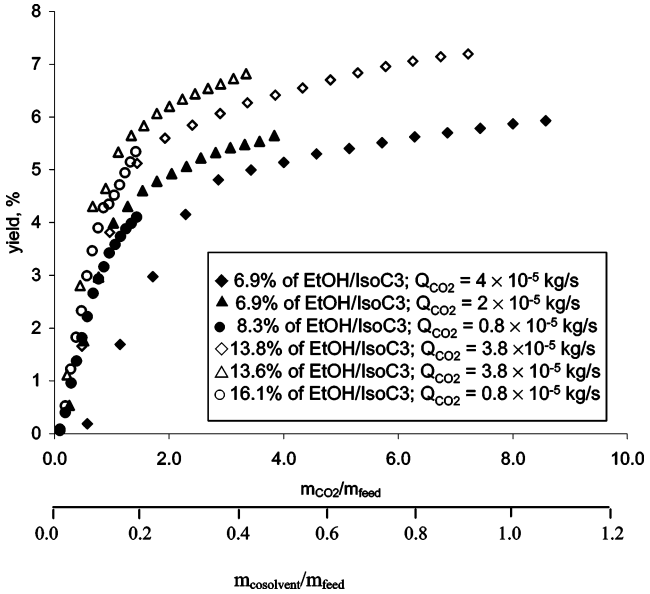

Figure 2. OECs for several operating conditions; SFE done at 300 bar and 30 °C.

The "not analyzed fraction" in **Table 1** contains starch degradation products formed during the drying process, such as oligosaccharides that were not analyzed. The high contents of starch and protein in the turmeric bagasse indicate that this residue can find an application in food processing. One of the possibilities is to hydrolyze the starch using subcritical water and supercritical CO_2, to obtain oligosaccharides and other glucose derivatives and a novel application that has been successfully used for ginger bagasse (*32*).

The turmeric particles' true densities were 1301.1 and 1207.0 kg/m^3 for turmeric M and S, respectively. The porosity of the bed was kept constant at 0.603 ± 0.005 using a mean particle diameter of 0.690 ± 0.003 mm.

The spline fitting to the OECs (**Figure 1**), as previously described in the literature (*19*), quantitatively described the experimental data. Therefore, the effects of the pressure and of the type of cosolvents can be assessed using the kinetic parameters shown in **Table 2**. The total yield or yield at the end of the extraction process (R_{TOTAL}) and the content of curcuminoids (CC) are also shown in **Table 2**. At 200 bar, the kinetic parameters (M_{CER}, Y_{CER}, t_{CER}, and R_{CER}), as well as the total yield and the content of curcuminoids, were larger for the EtOH/IsoC3 cosolvent mixture; intermediate values were observed for IsoC3, while the smaller values were obtained for EtOH. Nonetheless, the largest values for the kinetic parameters were obtained at 300 bar using IsoC3, followed by the EtOH/IsoC3 mixture and EtOH. This behavior indicates that the retrograde phenomenon was observed at the conditions used in this study. The solubility of a solute in a supercritical solvent and the global yield (R_{TOTAL}) are influenced by two phenomena: (i) the solute vapor pressure and (ii) the solvent density. The presence of a cosolvent will contribute to a more complex behavior of the system. Thus, at 200 bar, the interactions of the type solute/CO_2/IsoC3 were stronger than that of the type solute/CO_2/EtOH and predominated even for the cosolvent mixture EtOH/IsoC3. At 300 bar, the interactions of the type solute/CO_2/EtOH are probably responsible for the decrease in the kinetic parameters, but it positively affected the total yield and the curcuminoids content.

An ANOVA done for data in **Table 2** demonstrated the effects that the type of cosolvent significantly affected ($p_{value} < 0.10$) all kinetic parameters, except the total yield (R_{TOTAL}, in **Table 2**). The effects of pressure on the kinetic parameters were not significant ($p_{value} > 0.10$) except for R_{CER} ($p_{value} = 0.06$).

On the basis of the results of **Table 2**, a new set of experiments were done using the cosolvent mixture that maximized the global yield (R_{TOTAL}) and the content of curcuminoids (CC). The OECs are in **Figure 2**, and the kinetic parameters are in **Table 3**. Comparing the first two data arrays in **Table 3**, it is observed that doubling the amount of cosolvent increased the yield from 4.9 to 5.9% (wt) (increase of ∼20%) and the curcuminoids content from 0.003 to 0.04% (13-fold increase). Doubling the solvent flow rate and keeping the amount of cosolvent approximately constant (the second and third data arrays of **Table 3**) produced a huge increase in the content of curcuminoids, a result consistent with the total amount of solvent mixture (6.6×10^{-3} kg of CO_2 + 29.25×10^{-3} kg of EtOH/IsoC3). Increasing the solvent flow rate even further, but keeping the amount of cosolvent constant, negatively affected the content

Table 2. Operational Conditions and Kinetic Parameters for SFE Assays Using Turmeric M[a]

ϵ	$Q \times 10^5$ (kg/s)	cosolvent	$M_{CER} \times 10^7$ (kg/s)	$Y_{CER} \times 10^7$ (kg/s)	t_{CER}/60 (s)	R_{CER} (%)	R_{TOTAL} (%)	CC (%)
			pressure of 200 bar $\rho_{CO2} = 890.7$ kg/m^3[b]					
0.66	4.7	EtOH	5.2	1.8	135	4.1	5.8	0.0005
0.63	4.2	IsoC3	7.6 ± 0.3	3.0 ± 0.1	105 ± 8	4.3 ± 0.5	5.7 ± 0.3	0.0007
0.67	4.4	EtOH/IsoC3	7.8	3	111	4.5	5.9	0.0083
			pressure of 300 bar $\rho_{CO2} = 948.3$ kg/m^3[b]					
0.66	4.1	EtOH	3.6	1.4	210	4.3	5.8	0.0006
0.57	4.2	IsoC3	7.7 ± 0.6	3.5 ± 0.4	131 ± 26	4.8 ± 0.2	8.0 ± 0.4	0.0041
0.65	3.5	EtOH/IsoC3	6.3	3.0	138	4.8	7.8	0.0152

[a] Raw material = M; mean particle diameter = 0.7 mm; 30 °C; $Q_{CO_2} = (4.2 \pm 0.4) \times 10^{-5}$ kg/s; 1300 kg/m^3 (true density); (6.4 ± 0.6) % (v/v) of cosolvent. [b] Carbon dioxide density.

Table 3. Kinetics Parameters for the Assays Performed with Raw Material S at Various Solvent Flow Rates and Amounts of Cosolvent[a]

$Q \times 10^5$ (kg/s)	$m_{CO_2}(t_{CER}) \times 10^3$ (kg)	cosolvent (%)	$M_{CER} \times 10^7$ (kg/s)	$Y_{CER} \times 10^7$ (kg/s)	t_{CER}/60 (s)	R_{CER} (%)	R_{TOTAL} (%)	CC (%)
0.8	83	8.3	3.	6.6	192	2.8	4.9	0.003
0.8	82	16.1	3.7	8.6	200	3.9	5.9	0.04
1.9	88	13.6	11	10.2	88	4.6	7.9	0.12
2.0	119	6.7	7.6	6.7	108	4.1	6.3	0.01
3.8	160	13.8	11.8	5.6	81	5.2	7.8	0.06
4.0	254	6.9	8.7	3.7	112	4.5	6.3	0.04

[a] Raw material = S; T = 30 °C; P = 300 bar; cosolvent = 1:1 (v/v) EtOH/IsoC3; 1210 kg/m^3 (true density); ϵ = 0.54; average particle diameter of 0.69 mm.

Table 4. Yields and Curcuminoids Content Obtained by HD and Soxhlet for Turmeric and Turmeric Bagasse[a]

	yields (dry basis) (%)					curcuminoids content (dry basis) (%)				
	EtOH/IsoC3				EtOH	EtOH/IsoC3				EtOH
solvent	LPSE		Soxhlet			LPSE		Soxhlet		
raw material	1:100	1:10	1:100	1:10	1:100	1:100	1:10	1:100	1:10	1:100
M	9 ± 1	8.15 ± 0.02	21 ± 1	17 ± 2	21 ± 1	2.1 ± 0.5	0.34 ± 0.04	4.38 ± 0.02	5 ± 1	0.42
S	13 ± 2	7.5 ± 0.4	16 ± 1	15 ± 5	27 ± 2	3.1 ± 0.2	0.50 ± 0.01	8.43 ± 0.16	6.3 ± 0.7	0.38
R-M[a]			9.4 ± 0.1		26 ± 5			3.7 ± 0.4		1.4 ± 0.2
R-S[a]			10.3 ± 0.2		31 ± 12			3.9 ± 0.5		6.0 ± 0.8

[a] R-M and R-S mean turmeric bagasse from raw materials M and S, respectively, which resulted from the SFE performed using CO_2 and the cosolvent mixture of EtOH/IsoC3; solvent flow rates were 4.4×10^{-5} and 3.5×10^{-5} kg/s for raw materials M and S, respectively.

of curcuminoids (compare the third and the fifth data array of **Table 3**), although the total yield was approximately constant. This trend can be explained by considering that as the flow rate increased, the residence time of the solvent inside the extraction cell decreased, thus the time allowed for the interactions of the type solute/solvent decreased. Therefore, if diffusive phenomena are associated with the extraction of curcuminoids, which can happen depending on the location of curcuminoids rich cells, then the solvent flow rate should be carefully selected.

Table 4 shows the yields and the CC content obtained by the conventional processes of LPSE and Soxhlet extraction. The HD yields were 2.1 ± 0.5 (wt, dry basis) and 3.1 ± 0.5% (wt %, dry basis), for turmeric M and S, respectively; only traces of curcuminoids were detected in the HD extracts ($\sim 2 \times 10^{-4}$% [wt, dry basis]). The data in **Tables 2**−**4** show that the largest yield was obtained for turmeric S using the Soxhlet, EtOH, and feed to solvent ratio of 1:100. The smallest yield was determined for the raw material M in the HD process. Soxhlet extraction from turmeric S bagasse resulted in a yield of 31% (wt). Because of the low CC% in the SFE extracts, the CC% was considerably high in the turmeric bagasse Soxhlet extract. Nevertheless, increasing the feed to cosolvent ratio increased both the yields (R_{TOTAL} in **Table 3**) and the CC content; yet, the amount of cosolvent used was 10−100 times smaller than the amount of solvent used in the Soxhlet and LPSE processes. This explains the low CC content in the SFE extracts, despite the comparable yields of the SFE and $LPSE_{1:10}$ extracts.

TLC of the extracts showed that the Soxhlet extracts contained high molecular weight compounds as compared to the SFE extracts. **Table 5** shows the composition of the SFE, HD, LPSE, and Soxhlet extracts. The composition of the Soxhlet (1:100) was entirely different from the other extracts. The compositions of the HD and LPSE extracts were similar in chemical composition to the SFE extracts. For the SFE extracts, the relative proportions of ar-turmerone, (*Z*)-γ-atlantone, and (*E*)-γ-atlantone, the three major compounds detected in the turmeric volatile oil, represented ~60% (area %) of the extracts. This remained approximately constant for all conditions tested. However, the relative proportions of these three compounds varied within each condition. For SFE using EtOH, the relative proportions of the three compounds varied from 28 (ar-turmerone) to 20% (*E*)-γ-atlantone). For the extracts obtained with IsoC3, the relative proportion of (*Z*)-γ-atlantone reached 39.5% at 300 bar, and the relative proportions of the other two compounds (ar-turmerone and (*E*)-γ-atlantone) were approximately constant (15−18%). For the other substances, a small increase in the relative proportions with pressure was observed; the largest relative proportion of (*Z*)-γ-atlantone was detected at 300 bar. The behavior of the SFE extracts obtained using the EtOH/IsoC3 mixture was similar to that of the CO_2/IsoC3. The relative proportion of (*Z*)-γ-atlantone in the HD extract was 1−2 times larger than that of the SFE extracts.

The effects of the extraction kinetics (solvent flow rate) and the cosolvent amount on the composition of the SFE extracts are presented in **Table 6**. As expected, the solvent flow rate did not affect the composition of the SFE extracts. Data in **Tables 2**−**4** show that the amount of cosolvent or solvent (LPSE and Soxhlet) affected appreciably the yields and the CC content, but the same trend was not observed for the light fraction in the SFE extracts (the fraction that contains the compounds quantified by GC-FID).

The AAs of the turmeric extracts, expressed as % of inhibition of oxidation, are in **Table 7**. The SFE conditions slightly affected the AA, which varied from 15 to 25% of inhibition of oxidation for the first hour of reaction, increasing up to 28 and 43% after 3 h of oxidation reaction. Despite the absence of curcuminoids in the HD extracts, their AAs were considerably elevated as compared to the SFE extracts, but their AAs decreased over long reaction periods. The $Soxhlet_{1:100}$ extracts were very effective (elevated AA) for short reaction periods, and the AA slightly decreased for long reaction periods. The $LPSE_{1:100}$ extracts were the most effective antioxidant, since their AAs were larger than 60% of inhibition of oxidatio and increased up to 70% after 3 h of reaction. However, the ratio of solid to solvent used was too high (1:100) for commercial utilization. A comparison of the AAs for the $Soxhlet_{1:100}$ and the $LPSE_{1:100}$ with the data in **Tables 4** and **5** shows that a

Table 5. Composition of the Turmeric Extracts (Volatile Fraction) Obtained by SFE, HD, Soxhlet, and LPSE[a]

	process								
	SFE						HD	Soxhlet	LPSE
	EtOH		IsoC3		EtOH/IsoC3			(1:100)	
	pressure (bar)								
	200	300	200	300	200	300			
substances	relative proportion (area %)								
α-pinene	tr	tr	tr	tr	tr	tr	2.7		
1,8-cineole	tr	tr	0.18	tr	tr	tr	1.4		
trans-caryophyllene	tr	tr	0.3	0.4	0.5	0.4	tr		tr
Ar-curcumene	2.3	2.0	0.8	1.0	0.9	0.9	1.0		1.5
α-zingiberene	1.6	1.9	2.0	2.4	2.1	2.2	2.4		2.5
β-bisabolene	tr	0.5	0.3	0.4	0.5	0.4	tr		0.7
β-sesquiphellandrene	2.4	2.6	1.7	2.0	1.9	1.9	1.9		2.9
Ar-turmerol	1.2	1.0	0.7	0.8	0.8	0.7	1.1	6.5	tr
Ar-turmerol isomer	1.3	1.1	0.8	0.8	0.8	0.8	0.7		tr
Ar-turmerone	28.1	26.9	15.4	15.1	15.6	15.0	18.0		21.6
(*Z*)-γ-atlantone	24.2	24.7	35.4	39.5	35.1	36.2	44.0		33.4
(*E*)-γ-atlantone	20.3	19.8	16.9	18.0	17.2	17.1	18.3		18.7
dihydro-Ar-turmerone	tr	tr	0.4	0.4	tr	0.4	tr		tr
1-epi-cubenol	tr	0.7	0.6	0.6	0.6	0.6	0.6		tr
6*S*,7*R*-bisabolone	1.18	1.1	0.9	0.9	0.9	0.9	0.6		0.8
(*Z*)-α-atlantone	tr	0.5	0.5	0.4	0.5	1.7	0.6	17.0	0.8
(*E*)-α-atlantone	tr	tr	0.6	0.7	0.6	0.9	0.6		0.3
ni <	14.8	15.6	21.9	16.1	21.2	19.3	4.9	76.5	16.8

[a] tr = % < 0.34; ni = nonidentified.

Table 6. Composition of the Turmeric Extract as a Function of Flow Rate and Cosolvent Percent Obtained by SFE at 30 °C, 300 Bar, and Different Conditions of Process[a]

	relative proportion (area %) at					
solvent flow rate (kg/s)	3.8×10^{-5}	4.0×10^{-5}	1.9×10^{-5}	2.0×10^{-5}	0.8×10^{-5}	0.8×10^{-5}
cosolvent (%)	13.78	6.95	13.59	6.66	16.11	8.32
	substances					
α-pinene	tr	tr	tr	tr	tr	tr
1,8-cineol	0.91	tr	tr	tr	tr	tr
trans-caryophyllene	tr	tr	tr	tr	tr	tr
Ar-curcumene	1.04	1.09	1.01	0.91	0.99	1.02
α-zingiberene	2.11	1.88	2.22	2.04	2.25	2.34
β-bisabolene	tr	tr	tr	tr	tr	tr
β-sesquiphellandrene	2.01	1.82	1.97	1.93	2.12	2.19
Ar-turmerol	1.21	1.00	tr	1.03	1.00	0.99
Ar-turmerol isomer	tr	tr	tr	0.68	tr	tr
Ar-turmerone	20.17	18.25	19.32	17.703	17.91	17.68
(*Z*)-γ-atlantone	44.50	41.53	44.16	41.52	42.59	42.95
(*E*)-γ-atlantone	22.13	20.97	22.13	20.68	21.02	21.02
dihydro-Ar-turmerone	tr	tr	tr	tr	tr	tr
1-epi-cubenol	tr	tr	tr	tr	tr	tr
6*S*, 7*R*-bisabolone	1.04	1.07	1.07	1.09	1.03	0.98
(*Z*)-α-atlantone	0.92	tr	tr	tr	tr	tr
(*E*)-α-atlantone	1.47	tr	tr	0.64	tr	tr
ni <	3.49	12.39	8.12	11.77	11.09	10.83

[a] tr = % < 0.64; ni = nonidentified.

similar AA was detected despite the differences in CC content and volatile fraction composition. Therefore, the AAs of these extracts may be related to other compounds that were not detected by the analytical tools used (CG-FID and UV) in the present work to analyze the extracts.

Turmeric extracts have AC that begins at 0.25 μg/mL and exhibits cytostatic and cytolytic effects (*27*); the concentration at which the cytolytic effect began was specific for each cell line. **Figure 3** shows the results for the anticancer tests performed with the turmeric volatile oil (HD). Because the extract is considered to be active if its inhibition of growth is >50%, a dashed line was placed in the figure. As can be observed, the cytolytic effect of the HD extract started at 25 μg/mL for the breast (MCF-7) and ovary (OVACAR); for the breast expressing the multidrug resistance phenotype (NCIADR), the cytolytic effect started at an extract concentration of 250 μg/mL. Therefore, the turmeric volatile oil AC was lower than that of the SFE extracts (*27*); nonetheless, it was selective. The ACs of the curcuminoids mixture used as standard were not specific and started at 25 μg/mL, except for the lung cell (NCI.460), for which the cytolytic effect was observed at this concentration.

From these results, it is seen that a selection of the process to obtain turmeric extracts is dependent on the proposed use of the turmeric extracts. Despite the larger yields detected for the Soxhlet process, the light fraction was lost during processing (**Table 5**). On the other hand, the LPSE and the SFE processes produced extracts of similar composition, but LPSE was shown

Table 7. AA of *C. longa* Extracts Obtained with Different Methods

extract identification	inhibition of oxidation (%) at 1 h	2 h	3 h
raw material M			
SFE: cosolvent/pressure (bar)			
EtOH 200 bar	20	28	32
EtOH 300 bar	20	27	30
IsoC3 200 bar	23	34	39
IsoC3 300 bar	24	35	41
EtOH/IsoC3 200 bar	23	37	43
EtOH/IsoC3 300 bar	25	38	43
HD	43	31	25
Soxhlet EtOH/IsoC3 (1:100)	60	56	53
CC (standard)	43	39	39
LPSE EtOH/IsoC3 (1:100)	65	69	70
raw material S			
SFE: solvent flow rate (kg/s), % EtOH/IsoC3			
0.8×10^{-5}, 8.32	15	23	28
0.8×10^{-5}, 16.11	19	29	35
2.0×10^{-5}, 6.66	19	28	33
1.9×10^{-5}, 13.59	18	29	35
4.0×10^{-5}, 6.95	17	27	33
3.8×10^{-5}, 13.78	19	32	39
HD	44	33	28
Soxhlet, EtOH/IsoC3 (1:100)	56	50	47

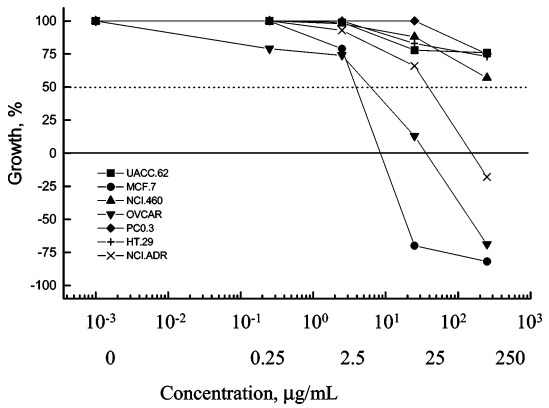

Figure 3. AC as a function of cancerous cellular ancestries for turmeric essential oil: UACC.62 (melanoma), MCF.7 (breast), NCI.460 (lung), OVCAR (ovary), PC0.3 (prostate), HT.29 (colon), and NCI.ADR (breast expressing the multidrug resistance phenotype).

to be in advantage with respect to the SFE process with respect to the yield of the CC%. Nevertheless, the LPSE and Soxhlet processes will require more solvent, and so, the SFE process is advantageous compared to them, when the costs of elimination of the cosolvent or of the solvent are considered. At this point, none of the studied processes should be abandoned. However, a cost of manufacturing analysis would be required in order to select the appropriate and economical process.

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