# OPTIMIZATION OF THE AR-TURMERONE EXTRACTION FROM TURMERIC (*Curcuma longa* L.) USING SUPERCRITICAL CARBON DIOXIDE

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## ABSTRACT

Extracting volatile compounds using supercritical carbon dioxide ($scCO_2$) is one of the most interesting applications of supercritical technology, due to the high solubility of these substances in $scCO_2$. Supercritical carbon dioxide extraction (SFE-$CO_2$) has been applied for obtaining extracts from several vegetable matrices, among them turmeric (*Curcuma longa*), because its volatile oil contains a target bioactive compound: ar-turmerone. However, the optimization of the SFE-$CO_2$ operating conditions aiming to obtain ar-turmerone rich extract has not been studied yet. Therefore, applying supercritical technology for optimizing ar-turmerone extraction from C. *longa* L was the objective of this study. Turmeric rhizomes were ground, sieved and placed in contact with $scCO_2$ flowing at $8.5\times10^{-3}$ kg/min in a laboratorial-scale SFE unit. An experimental full factorial design composed of six levels of pressure (10, 15, 20, 25, 30 and 35 MPa) and three levels of temperature (313, 323 and 333 K) was carried out in duplicate for evaluating the global and ar-turmerone yields. From these assays, we selected three conditions (333K/25MPa, 333K/20MPa and 313K/20MPa) presenting higher yields to study the kinetics of the process. Major compounds in the extracts were identified and quantified by gas chromatography. Using SFE-$CO_2$ led to high extract and ar-turmerone yields. Fost extraction combined with relatively low solvent consumption were observed. According to the spline model, solvent to feed mass ratio of only 0.94 would be required for extracting more than 90% of the ar-turmerone contained in the raw material at 333 K and 20 MPa. Thus, we suggested that this is a suitable condition for obtaining ar-turmerone.

## INTRODUCTION

Supercritical carbon dioxide extraction (SFE-$CO_2$) of volatile compounds is one of the most effective applications of the supercritical technology. The high solubility of these substances in supercritical carbon dioxide ($scCO_2$) medium and the facility in modifying the selectivity of the compounds of interest through changes in the process variables are some important aspects that enable the application of this technology [1]. Furthermore, the solvent can be easily removed from the mixture by pressure reduction.

Turmeric is the rhizome of the plant *Curcuma longa* L., a tropical herb of the zingiberaceae family and native to southern Asia. The turmeric volatile oil is rich in sesquiterpenes compounds. Among these, ar-turmerone is of great interest due to its use in the prevention and treatment of various diseases. Its bioactivity has been associated with anti-inflammatory, antioxidant, antimicrobial and anticarcinogenic [2-5] properties. Some

literature works have reported the optimization of oil extraction from *C. longa* using $scCo_2$ [6] and $scCo_2$ plus cosolvent [7]. The oil extraction by SFE-$CO_2$ and the extract purification into two fraction (ar-turmerone-rich and α-β-turmerone-rich fractions) by liquid-solid chromatography has been evaluated in another work [8]. Furthermore, a more recent work tested the phase equilibrium of a pseudo-compound named α-β-ar-turmerone and composed of three turmerones (α- turmerone, β- turmerone, and ar-turmerone) in $scCO_2$ [9]. However, the optimization of the extraction conditions to obtain ar-turmerone rich extract have not been reported in literature.

In the work reported here, the optimization of ar-turmerone extraction using SFE-$CO_2$ was investigated. The first step of the work consisted of the construction of the Global Yield Isotherms (GYI). These curves enable to select the conditions of temperature and pressure [10, 11] that result in higher yield of the target compounds. Later, Overall Extraction Curves (OEC) were obtained in the defined conditions of temperature and pressure and evaluated based on the kinetic parameters calculated by fitting the experimental data to a *spline* [12].

## MATERIALS AND METHODS

### Raw material characterization and preparation

Turmeric rhizomes (*Curcuma longa* L.) were obtained from Oficina de Ervas Farmácia de Manipulação Ltda (lot 065DM, Ribeirão Preto, Brazil). The rhizomes were collected in September 2012 and were held under freezing for 10 days. Later, they were dried under shade until moisture of 8 %, stored in plastic bags and kept in a domestic freezer at 263 K (Metalfrio, model DA420, São Paulo, Brazil). Before the assays, the rhizomes were ground in a knife mill (Marconi, model MA340, Piracicaba, Brazil) using sieve with opening of 1.5 mm. The ground raw material was classified according to the particle size using a vibratory system (Bertel, model 1868, Caieiras, Brazil) assembled with 8–100 mesh sieves (Tyler series, Wheeling, USA). The particle mean diameter ($d_p$) was determined according to the ASAE Standards [13]. The moisture content of the raw material was determined by the xylene distillation method [14], indicated to raw material rich in volatile oil. The true density of the particles ($\rho_r$) was determined by picnometry with helium gas (Quantachrome Instruments, model Automatic Pycnometer Ultrapyc 1200e, Boynton Beach, USA) at the Analytical Center of the Institute of Chemistry, University of Campinas (Campinas, Brazil). The apparent density of the bed ($\rho_a$) was calculated by dividing the sample mass loaded into the extraction cell by cell internal volume. The total porosity of the bed ($\varepsilon$) was calculated as: $\varepsilon = 1 - (\rho_a/\rho_r)$.

### SFE-$CO_2$ procedures

Laboratorial-scale SFE unit, named SFE-I [15] and equipped with a 415-$cm^3$ extraction cell (3.14 cm diameter and 46 cm height, internal dimensions) was used to perform the SFE assays in order to obtain the GYIs and OECs from turmeric. The raw material sample was placed inside the extraction vessel with the aid of a nylon cell presenting approximately the same diameter as the vessel. To fill the extraction vessel completely, the empty space of the vessel was filled with glass beads of meshes 8 – 10 and a Teflon column. The temperature control was performed using a thermostatic bath (Marconi, model 159/300, Piracicaba, Brazil) and the pressure system was kept by an air-driven pump (Maximitor GmbH, model M111, Nordhausen, Germany) and a back pressure regulator valve (Tescom Corporation, model 26-171, Elk River, USA). The extracting solvent was carbon dioxide (99.9 % purity, Gama Gases, São Bernardo do Campo, Brazil). The GYIs were obtained based on a full factorial design composed of six levels of pressure (10, 15, 20, 25, 30 and 35 MPa) and three levels of

temperature (313, 323 and 333 K) and carried out in duplicate. For these runs the solvent (S) to feed (F) mass ratio was maintained constant at 12.1.

The OECs were constructed in duplicate and the defined extraction conditions based on the GYIs results were 313 K / 20 MPa, 333 K / 20 MPa and 333 K / 25 MPa. The kinetics parameters were estimated from the spline model [16] with 2 straight lines using the Proc Reg and the Proc Nlin procedures of SAS 9.2® [17]. The first line represents the constant extraction rate period (CER) and the second the diffusion controlled period (DC). The following kinetic parameters were obtained for the CER period as described by Meireles [11]: mass-transfer rate ($M_{CER}$) represented by the slope of the first line; length of the CER period ($t_{CER}$) corresponding to the interception of the first and second lines; mass ratio of solute in the supercritical phase at the column outlet ($Y_{CER}$) obtained by dividing $M_{CER}$ by the mean solvent flow rate for the CER period; and yield relative to the CER period ($R_{CER}$). Moreover, the solvent (S) to feed (F) mass ratio, relative to the CER period ($S/F_{CER}$), was also calculated. All experimental data are presented in Table 1. Statistical analyzes of ANOVA and Tukey test were carried out using the commercial software Minitab® version 16.

**Table 1:** Bed characterization and operational data of experiments.

| | Hydrodistillation | SFE-$CO_2$ | |
|---|---|---|---|
| | | GYIs | OECs |
| *Raw material characterization* | | | |
| Moisture (%) | 13 ± 0.5 | 13 ± 0.5 | 13 ± 0.5 |
| $d_p$ ($10^{-4}$ m)[a] | 6.75 ± 0.04 | 6.75 ± 0.04 | 6.75 ± 0.04 |
| $\rho_t$ (kg/m$^3$)[b] | 1450 ± 10 | 1450 ± 10 | 1450 ± 10 |
| *Experimental data* | | | |
| F (g)[c] | 25 | 47 | 76.4 |
| $\rho_a$ (kg/m$^3$)[d] | - | 840 ± 21 | 840 ± 21 |
| Porosity | - | 0.42 ± 0.01 | 0.42 ± 0.01 |
| Static period (min) | - | 20 | 20 |
| *Extraction parameters* | | | |
| $Q_{CO2}$ ($10^{-3}$ kg/min)[e] | - | 8.6 ± 0.2 | 8.4 ± 0.4 |

Results are presented as mean ± standard deviation

[a]Mean particle diameter ($d_p$); [b]true density of the particles ($q_r$); [c]raw material mass (F), approximately value; [d]apparent density of the bed ($q_a$); [e]$CO_2$ flow rate ($Q_{CO2}$).

**Chemical composition of the extracts**

The turmeric extract compositions were determined in a gas chromatograph with flame ionization (GC-FID) (Shimadzu, CG 17A, Kyoto, Japan) system equipped with a fused-silica capillary column DB-5 (J&W Scientific, 5% phenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm, Folsom, USA). The operating conditions and procedure of analysis were adapted from the work of Braga et al. [7] in short: dilution of samples in ethyl acetate (Merck, analytical standard, Darmstadt, Germany) using an approximate ratio of 5 mg of extract per cm³ of solvent; helium (99.9 % purity, White Martins, Campinas, Brazil) as carrier gas at a flow rate of 1.4 cm³/min; split injection conducted with injection volume of 1 μL and split ratio of 1:30; injection temperature of 513 K; initially column temperature of 393 K, then programmed at 2 K/min to 453 K, then at 10 K/min to 503 K and held for 5 min; detection temperature of 553 K.

The turmeric volatile compounds were identified by Gas Chromatograph-Mass Spectrometry (GC-MS) at the Analytical Center of the Institute of Chemistry, University of Campinas (Campinas, Brazil). GC-MS were performed on a gas chromatography instrument

(Agilent, 5975C Series GC/MSD System, Santa Clara, USA) equipped with a capillary column HP-5MS (Agilent J&W, 5% phenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d. × 0.25 µm, Santa Clara, USA) and coupled with a mass spectrometer. The operational conditions of compound separations were identical to those previously described for GC-FID. The mass spectrometer was operated in electron-impact mode, the scan range was 40-500 Daltons, the ionization energy was 70 eV and the scan rate was 0.5 s/scan. The obtained mass spectra were compared with the NIST 11 mass spectral database [18] and spectral informations from literature [19, 20]. The quantification of the three turmerone (ar-turmerone, α-turmerone e β-turmerone) was performed using ar-tumerone (Sigma-Aldrich, (S)-ar-Turmerone analytical standard, St. Louis, USA) standard calibration curve. The α- and β-turmerone quantification based on the ar-turmerone calibration curve can be justified by the great similarity among these compounds.

## RESULTS

### Identification of turmeric compounds

In Figure 1 typical GC-FID chromatogram of turmeric extract obtained by SFE-$CO_2$ is presented. About 30 compounds were detected in the extracts. The main compounds were (1) ar-curcumene, (2) zingiberene, (3) β-sesquiphellandrene, (4) ar-turmerone, (5) α-turmerone e (6) β-turmerone (also known as curlone [21]). Qin et al. [20] obtained similar chromatographic profile of *C. longa* Jianghuang extract obtained by pressurized liquid extraction. The extracts obtained here presented high content of the three turmerones (ar-, α- and β-turmerone). Together they represented on average 75 % of the extracts. Only the ar-turmerone, the main bioactive compound of the plant volatile oil, represented almost 20 % of the extracts.

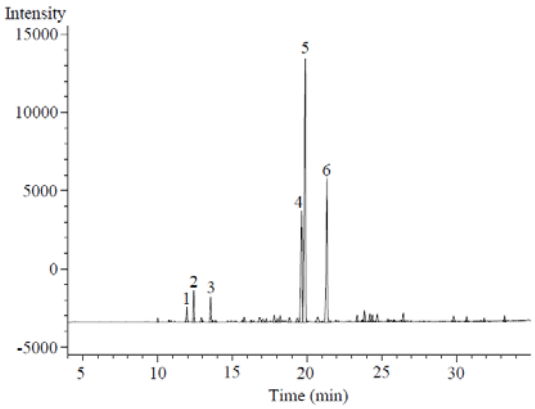


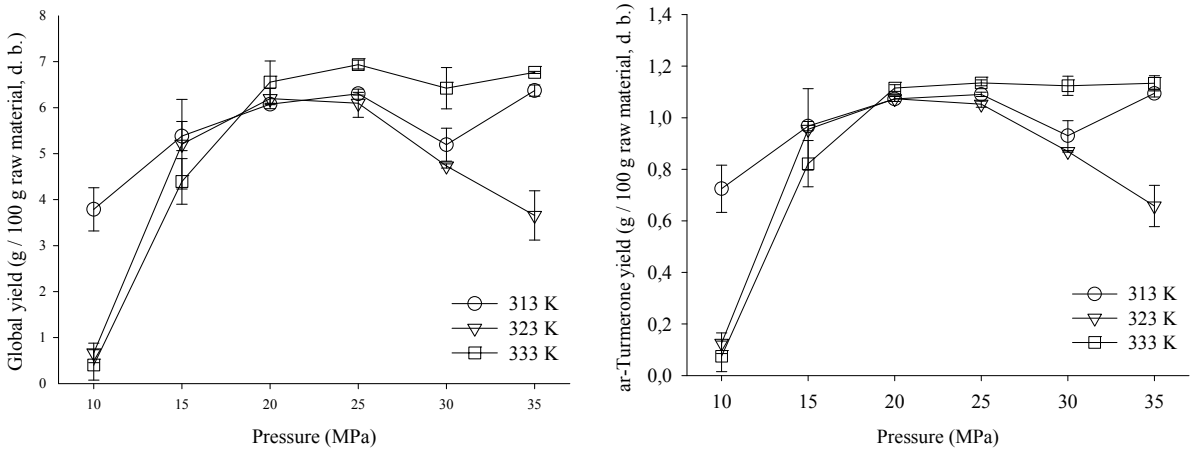

**Figure 1:** GC-FID chromatogram of *C. longa* L. obtained by SFE-$CO_2$ (333 K, 25 MPa, and $8.6 \times 10^{-3}$ kg/min).

### Global Yield Isotherms

Global yield and ar-turmerone yield isothermal curves are presented in Figures 2a and 2b, respectively. The variation of ar-turmerone yield with pressure and temperature was similar to that of global yield. Moreover, the extract compositions varied slightly among the process conditions analyzed.

For the pressure range studied (10 -35 MPa), the isotherms presented crossover region near to 20 MPa. The inversion pressure of the isotherms is the result of the phenomenon known as retrograde condensation [22]. This phenomenon is characterized by high solubilities

of the solutes at low temperatures before the inversion point (pressure). On the other hand, high solubilities at high temperatures are observed when using larger pressures than that of inversion (Figures 2a and 2b) [23]. This event can be roughly explained by a balance between two effects. The first is the combined effect of the temperature and pressure on the solvent density. The second is the variation of the solute vapor pressure with the temperature. In low pressures (here between 10 and near to 20 MPa) small variations in the temperature and/or pressure presented high influence on the solvent density [24]. Increasing temperature in the system led to the decreasing of the solvent density and, consequently, the solubility of the compounds dropped down in the solvent. Further, the solubility of the compounds increased rapidly with a pressure change from 10 to 20 MPa. As it can observed in Figures 2a and 2b, for pressures larger than that of inversion the solvent density drop with the temperature increase is not as important as at low pressures. The density drop was overlapped by the vapor pressure increase of the solutes [25]. This was true for the yields obtained at 333 K in comparison with those obtained at 313 and 323 K. The similarities among the isotherms shown in Figures 2a and 2b indicates that the process conditions did not greatly affected the composition of the extracts; this was also observed by Began et al. [6].




(a) (b)

**Figure 2:** Global yield (a) and ar-turmerone yield (b) isotherms of *C. longa* L. obtained by SFE-$CO_2$ (Q = 8.6 × $10^{-3}$ kg/min; S/F = 12.1).

Maximum extract yield of 6.9 % (kg extract / 100 kg dry raw material) and the highest relative yield in ar-turmerone, 1.14 % were obtained at 333 K and 25 MPa (Table 2). However, no significant differences were observed among these yields and those in the ranges of 6.1-6.9 % for the extract and 0.93-1.14 % for the ar-turmerone (Table 2) by the Tukey test (at 5% level of significance). At 333 K, the global and ar-turmerone yields remained relatively constant from 20 to 35 MPa (Figure 2a and 2b). When the process was performed at 313 and 323 K were observed a solubility drop of the compounds at 30 MPa and an even smaller solubility at 35 MPa e 323 K. Began et al. [6] identified 308 K and 22.5 MPa as the optimum condition for the extraction of turmeric volatile oil by SFE-$CO_2$ using response-surface methodology (RSM). Also by RSM, Chang et al. [8] found that values near to 320 K and 26 MPa are adequate to extract the three turmerones with purity close to 71%. In more recent work using SFE-$CO_2$, Kao et al. [9] obtained extraction yield of 6.98 wt% (weight percent) at 313 K and 26 MPa; the extract contained 67.7 % of turmerones (ar-, α- and β-turmerone). In the work reported here, extracts containing from 70.5 to 75 % of the three turmerones were obtained in the conditions of high yields.

**Table 2:** Global yields of extraction and ar-turmerome (%, g / 100 g dry raw material) as function of operating conditions

| Pressure (MPa) | Temperature (K) | | |
|---|---|---|---|
| | 313 | 323 | 333 |
| | Global yields | | |
| 10 | $3.8 \pm 0.5^{*fg}$ | $0.7 \pm 0.2^{h}$ | $0.4 \pm 0.3^{h}$ |
| 15 | $5.4 \pm 0.3^{bcde}$ | $5.2 \pm 0.1^{cdef}$ | $4.4 \pm 0.5^{efg}$ |
| 20 | $6.1 \pm 0.0^{abcd}$ | $6.2 \pm 0.2^{abcd}$ | $6.6 \pm 0.5^{abc}$ |
| 25 | $6.3 \pm 0.0^{abc}$ | $6.1 \pm 0.3^{abcd}$ | $6.9 \pm 0.1^{a}$ |
| 30 | $5.2 \pm 0.4^{cdefg}$ | $4.7 \pm 0.0^{defg}$ | $6.4 \pm 0.4^{abc}$ |
| 35 | $6.4 \pm 0.1^{abc}$ | $3.7 \pm 0.5^{g}$ | $6.8 \pm 0.0^{ab}$ |
| | ar-Turmerone yields | | |
| 10 | $0.72 \pm 0.09^{de}$ | $0.13 \pm 0.04^{f}$ | $0.07 \pm 0.06^{f}$ |
| 15 | $0.97 \pm 0.02^{abc}$ | $0.96 \pm 0.16^{abcd}$ | $0.82 \pm 0.09^{cde}$ |
| 20 | $1.07 \pm 0.01^{ab}$ | $1.07 \pm 0.01^{ab}$ | $1.12 \pm 0.01^{a}$ |
| 25 | $1.09 \pm 0.01^{ab}$ | $1.05 \pm 0.01^{abc}$ | $1.14 \pm 0.01^{a}$ |
| 30 | $0.93 \pm 0.06^{abcd}$ | $0.87 \pm 0.00^{bcde}$ | $1.12 \pm 0.04^{a}$ |
| 35 | $1.09 \pm 0.01^{ab}$ | $0.66 \pm 0.08^{e}$ | $1.13 \pm 0.03^{a}$ |

*Results are presented as mean ± standard deviation; a–h letters represent significant difference at 5% level of significance between the extraction conditions.

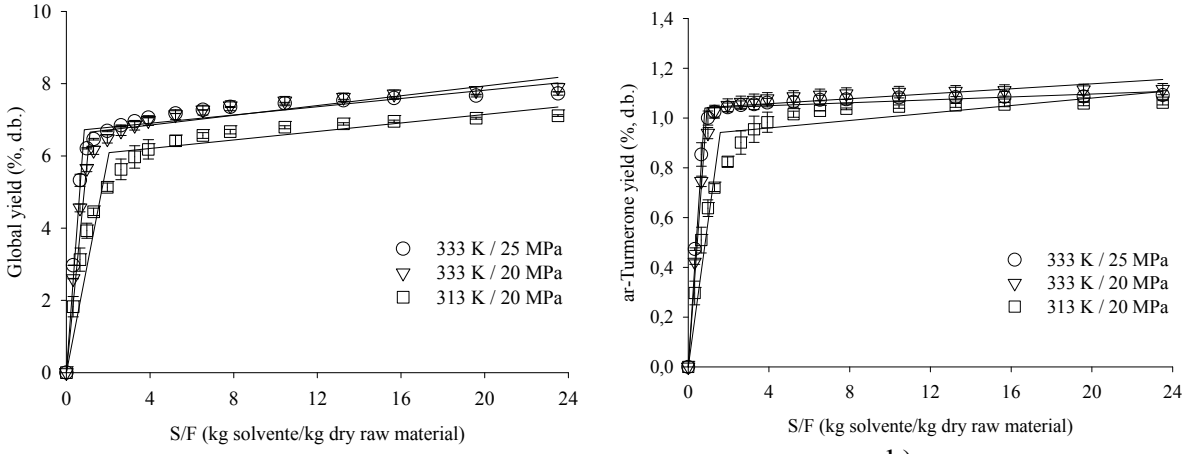
Now, in agreement with the results reported here and those cited from the literature, it seems clear that an extraction condition that includes pressure in the range of 20-25 MPa and temperature in the range of 313 – 333 K presents an adequate condition to obtain ar-turmerone-rich extracts. Pressures higher than 25 MPa (and up to 35 MPa) can be discarded, because it does not lead to an increase in the extraction of the target compounds and, on the other hand, can impact significantly in the operating cost of the SFE process.

**Overall Extraction Curves**

OECs for turmeric extracts were built in three conditions (temperature/pressure) (Figure 3a). These conditions were selected based on the results of the yield isotherms. The experimental OECs data were fitted to a spline with 2 straight lines and the kinetic parameters are presented in Table 3. The 333K/25MPa and 333K/20MPa conditions were equivalent, no significant differences were observed between their parameters of $S/F_{CER}$, $t_{CER}$, $R_{CER}$ and $R_T$ by the Tukey test (at 5% level of significance). However, comparing these conditions with 313K/20MPa significant differences can be noted with respect to $S/F_{CER}$, $t_{CER}$, e $R_T$. For 313K/20MPa about 2.0 kg solvent / kg dry raw material ($S/F_{CER}$) would be necessary to reach similar yields to those obtained at 333K/25MPa and 333K/20MPa in the end of CER period. In other words, the double amount of the solvent is required. The mass ratio of extract in the supercritical phase at the column outlet ($Y_{CER}$) presented high levels, in the range of $10^{-2}$ kg extract/kg solvent, allowing, for example, that $S/F_{CER}$ of only 1.1 was required to extract more than 80 % of extractable compounds at 333K and 20MPa.

Extraction kinetics for ar-turmerone from were also obtained (Figure 3b) and the kinetic parameters are presented in Table 3. Similar results to those discussed above were found. For the 333K/25MPa and 333K/20MPa conditions the kinetic parameters of $S/F_{CER}$, $t_{CER}$, $R_{CER}$ and $R_T$ did not present significant differences by the Tukey test (at 5% level of

significance). On the other hand, significant difference for $S/F_{CER}$ was observed comparing these two conditions with 313K/20MPa. The easy access to the solute by the solvent contributed to high yields associated with low solvent consumption. According to the spline model, $S/F_{CER}$ ratios of only 0.83 and 0.94 would be required to extract more than 90 % of the ar-turmerone contained in the raw material at 333K/25MPa and 333K and 20MPa, respectively.




a) b)

**Figure 3:** Extraction curves for extract (a) and ar-turmerone (b) at various experimental conditions by SFE-$CO_2$; (—) data fitted using SAS 9.2®.

**Table 3:** Kinetic parameters estimated by the spline model (using SAS 9.2®).

| Extraction condition | $t_{CER}/60$ (s)[A] | $S/F_{CER}$ (kg/kg)[B] | $M_{CER}\times10^6$ (kg/s)[C] | $Y_{CER}\times10^2$ (kg/kg)[D] | $R_{CER}$ (% d.b.)[E] | $R_T$ (%, d.b.)[F] |
|---|---|---|---|---|---|---|
| | Based on global yield | | | | | |
| 333 K / 25 MPa | $6.7 \pm 0.1^b$ | $0.9 \pm 0.0^b$ | $11.0 \pm 0.2^a$ | $7.9 \pm 0.1^a$ | $6.7 \pm 0.0^a$ | $7.7 \pm 0.0^a$ |
| 333 K / 20 MPa | $9.6 \pm 0.3^b$ | $1.1 \pm 0.0^b$ | $7.2 \pm 0.1^b$ | $5.9 \pm 0.0^b$ | $6.7 \pm 0.1^{ab}$ | $7.9 \pm 0.1^a$ |
| 313 K / 20 MPa | $15.6 \pm 1.3^a$ | $2.0 \pm 0.1^a$ | $3.7 \pm 0.1^c$ | $3.0 \pm 0.1^c$ | $6.1 \pm 0.2^b$ | $7.1 \pm 0.0^b$ |
| p-value | 0.003 | 0.002 | 0.000 | 0.000 | 0.040 | 0.004 |
| | Based on ar-Turmerone yield | | | | | |
| 333 K / 25 MPa | $6.48 \pm 0.09^b$ | $0.83 \pm 0.00^b$ | $1.76 \pm 0.07^a$ | $1.26 \pm 0.02^a$ | $1.04 \pm 0.02^a$ | $1.09 \pm 0.02^a$ |
| 333 K / 20 MPa | $8.02 \pm 0.24^{ab}$ | $0.94 \pm 0.02^b$ | $1.42 \pm 0.02^b$ | $1.10 \pm 0.01^b$ | $1.04 \pm 0.02^a$ | $1.12 \pm 0.02^a$ |
| 313 K / 20 MPa | $12.14 \pm 1.75^a$ | $1.59 \pm 0.21^a$ | $0.78 \pm 0.05^c$ | $0.60 \pm 0.06^c$ | $0.94 \pm 0.03^a$ | $1.06 \pm 0.01^a$ |
| p-value | 0.024 | 0.015 | 0.001 | 0.001 | 0.048 | 0.110 |

*Results are presented as mean ± standard desviation; a–c letters represent significant difference at 5% level of significance between the data. [A]Extraction time; [B]Solvent (S) to feed (F) mass ratio (kg solvent / kg dry raw material); [C]Extraction rate; [D]Solute mass ratio in the supercritical phase at the extractor exit (kg solute / kg solvent); [E]Extraction yield (%, dry basis). All about of CER period. [F]Experimental extraction yield after 3 hours (%).

Given what was presented, both with respect to ar-turmerone as to the extract, it seems that the 333K/25MPa and 333K/20MPa conditions exhibit advantages in solubility and mass transfer of the compounds in comparison to 313K/20MPa. Therefore, they have potential for a rapid extraction process combined with savings of the solvent. Furthermore, considering that for 333K/25MPa and 333K/20MPa no significant differences were observed in terms of $S/F_{CER}$ and $R_{CER}$, we can recognize that 333 K and 20 MPa are suitable for obtaining the extract and ar-turmerone. This condition led to high yield and extraction rate and presents lower impact in the operational cost of the process, due to the lower pressure.

## CONCLUSION

The turmeric rhizomes used in this study presented almost 20 % of ar-turmerone in the extracts obtained by SFE-$CO_2$ and about 75 % of the three turmerones (ar-, α- and β-turmerone, major compounds). The extract composition was slightly affected by the conditions studied. The use of SFE-$CO_2$ led to higher yields of extract and ar-turmerone. Moreover, fast extraction and relatively low solvent consumption were observed. Finally, we can recognize that temperature of 333 K and pressure of 20 MPa presents suitable for obtaining the extract and its bioactive compound ar-turmerone by SFE-$CO_2$.

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