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Extraction of Essential Oil and Pigments from *Curcuma longa* [L.] by Steam Distillation and Extraction with Volatile Solvents

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Curcuma longa [Linn] (turmeric), of the Zingiberaceae family, has a great importance in the food, textile, and pharmaceutical industries. The aim of this work was to identify the best processing conditions to maximize the yields of essential oil and pigments, as well as their content of *ar*-turmerone, (α and β)-turmerone, and the curcuminoids, respectively. Autoclave pressure and distillation time were the variables studied for the steam distillation process. The highest yields of essential oil (0.46 wt %) and pigment (0.16 wt %)—expressed as curcumin, demethoxycurcumin, and bisdemethoxycurcumin—were obtained at a pressure of 1.0×10^5 Pa and a time of 2 h. On the other hand, with extraction by volatile solvents, the best yield of essential oil (5.49 wt %) was obtained when using 0.175, 0.124, 0.088 mm particles (Foust, A. S.; Wenzel, L. A.; Clump, C. W.; Maus, L.; Andersen, L. B. *Princípios das Operações Unitárias*; Editora Guanabara Dois S.A.: Rio de Janeiro, Brazil, 1982), at 40 °C, and 6 h of extraction. However, the best yield of pigment (7.98 wt %) was obtained under the same conditions, except for the temperature (30 °C).

KEYWORDS: Volatile solvents; steam distillation; *Curcuma longa*

INTRODUCTION

The desire of consumers and industries worldwideto replace artificial pigments in foodstuffs has contributed to a growing interest in natural pigments. Although the food nutritional value of a pigment is more important than its visual properties, the combination of taste, flavor, texture, and color has an influence on food acceptability. Several studies have demonstrated that most natural pigments have other important properties besides being responsible for the food color. Curcumin obtained from rhizomes of *Curcuma longa* is considered to be one of the main pigments produced in Brazil. Besides the yellow pigment for food, this plant is widely used as a seasoning. Mature rhizomes are ground to give an aromatic yellow powder, employed as a coloring ingredient of "curry powder". Today, turmeric, turmeric extracts, and turmeric oleoresins are commercial products produced in large quantities in Brazil. With the demands for natural colors, the use of turmeric is likely to increase.

Turmeric has been widely used in Indian medicine due to its beneficial effects, such as bilious regulating functions (*2*, *3*), reducing cholesterol levels (*4*, *5*), and anti-inflammatory (*6*−*8*) and antiarthritic activities (*9*). In addition to the fact that the alcoholic extract can be used to inhibit microorganism development in cholecystitis (*10*), curcuminoids can be used as bacteriostatics against *Staphylococcus* (*11*), and the essential oils have shown bactericidal (*10*) and fungistatical activities (*12*). *ar*-Turmerone, which can be found in the essential oil of turmeric, has potential as an antivenon agent (*13*).

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A series of processes to obtain the active principles of plants has been investigated. Among the usual essential oil extraction processes, steam distillation (commercially, the most used in Brazil) and extraction by volatile solvents can be highlighted. The simplicity of the steam distillation technology, used for the extraction of active principles from plants, is an additional advantage to explore plant resources (*14*). The possibility to develop and improve the quality of the turmeric-derived products, having a higher value, represents an excellent market product for Brazil, as a rhizome producer. The quality of a turmeric-derived product is related to the presence of curcuminoids and its essential oil.

MATERIALS AND METHODS

Raw Materials. Turmeric rhyzomes (*C. longa* [L.]), planted in the region of Maringá, Paraná, and harvested after 9 months, were utilized.

Curcuminoids. Pure curcumin standard, indexed under no. 75300, E-100 (*15*), was acquired from Sigma-Aldrich Chemical Representações Ltda. (São Paulo, Brazil). Demethoxycurcumin and bisdemethoxycurcumin are homologues of curcumin and are sold together, having the same colorizing power (*16*). In **Figure 1** are shown the structures of the three curcuminoids contained in the standard.

Reagents. The reagents used were ethyl acetate of HPLC grade, 99.9% (Mallinckrodt, São Paulo, Brazil), and ethanol (Induslab, Arapongas, Brazil). All other chemicals were of analytical grade (Carlo Erba, São Paulo, Brazil).

Preparation of Turmeric Rhizomes for Steam Distillation. Turmeric rhizomes were harvested at once and dried in sunlight, until reaching 69% humidity. This drying method was chosen because there was no variation in the raw material properties compared to the drying

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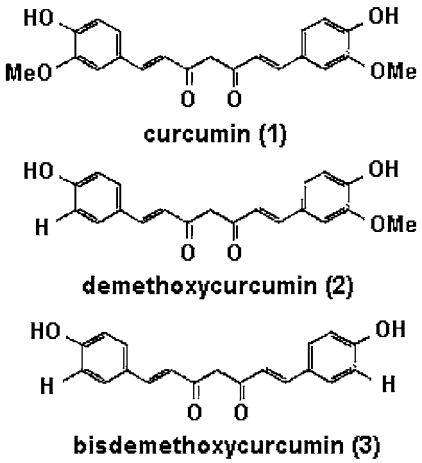

Figure 1. Structures of (**1**) curcumin, (**2**) demethoxycurcumin, and (**3**) bisdemethoxycurcumin.

process in an oven. Before each extraction, ≅1 kg of turmeric was triturated and mixed with the same quantity of glass beads. This procedure was used to prevent compaction of the material inside the distiller. After that, the material was put into the distiller.

Apparatus for Steam Distillation. The steam distillation apparatus has been previously described (*17*). The vapor was generated in an autoclave with pressure control. It was injected in the still containing an expansion camera, a perforated plate used to hold the basket with the raw material, and a condenser. After passing through the turmeric bed, steam was condensed by cooling water at room temperature, being collected, in a separation funnel, together with the essential oil obtained. The mixture was allowed to settle for 1 day; afterward, the essential oil was collected in small flasks closed with rubber lids wrapped with Teflon, weighed in an analytical scale balance standard apparatus, and kept under refrigeration for later chromatographic analysis. The lower valve of the distiller was opened only after the extraction, to collect pigment and other solids suspended in hot water (drain). The water collected at the drain was left in cold storage for a week so that suspended solid matter could settle.

After 1 week, the decantation water was siphoned off, and the solid was put into an oven at 40 °C to dry. After the solids had dried, high-performance liquid chromatography (HPLC) was used to measure the amount of curcuminoids contained in them. The amount of pigments (curcuminoids) dissolved in decantation water was determined by spectrophotometry.

Recovered oil was reported in grams of oil per gram of dried rhizomes (moisture of 69%).

Preparation of Turmeric Rhizomes for Extraction with Volatile Solvent. The turmeric rhizomes were harvested at once, ground, and dried in an oven with air recirculation at 50°C, until reaching a final humidity of 12%. The dried rhizomes were triturated using a model TE 340 knife mill (Tecnal, Jundiaí, Brazil). The particle size distribution was determined using sieves of the Tyler series (20, 28; 35, 48, 60, and 80, 115, 170 mesh) or their correspondent metric units (0.833, 0.589; 0.417, 0.295, 0.248, and 0.175, 0.124, 0.088 mm) and an agitator (Belter, Piracicaba, Brazil). For each particle size, the enzymatic degradation of starch was performed (*18*). Afterward, particles were dried again in an oven with air recirculation at 50 °C and their humidity was determined (10%). Due to the enzymatic degradation of starch, 0.833, 0.589; 0.417, 0.295, 0.248, and 0.175, 0.124, 0.088 mm particles lost 27.5, 24.7, and 30.2%, respectively, in starch and water.

Apparatus for Extraction with Volatile Solvents. After the material had been prepared, the extraction of turmeric essential oil was made using a model MA 830 shaker (Marconi, São Paulo, Brazil). The extractions were done in two batches, one at 20 °C and the other at 40 °C. The flasks (Erlenmeyer), covered with rubber corks, containing 4 g of triturated turmeric and 50 mL of petroleum ether, were weighed before and after the extraction to verify if there was a loss of solvent during the extraction. The mixture of solid and solvent was kept under agitation for the extraction time. Afterward, the mixture was vacuum filtered. The micelle (solvent plus oil) was placed in a dark flask, covered, weighed, and put inside on oven at 40 °C, the essential oil thus being obtained. Recovered oil was reported in grams of oil per gram of dried solid.

For pigment extraction, the same samples, dry and free of essential oil, were weighed again and put into Erlenmeyer flasks, with the addition of 40 mL of ethanol. The extractions were done in two batches, one at 30 °C and the other at 60 °C. The filtered materials were placed on Petri plates and kept in an oven at 40 °C to evaporate the ethanol.

After the separation, the pigment obtained was reported in grams of pigments per gram of dried solid (without essential oil); gas chromatography (essential oil), liquid chromatography, and spectrophotometry (pigments) were performed.

Factorial Designs. Two factorial designs were made, one for the steam distillation process and the other for the process of extraction with volatile solvents. The first one used two variables: distillation time (minutes) in two levels (DT = 1 and 2 h), and autoclave pressure (pascals), in three levels (AP = 1.0×10^5, 1.3×10^5, and 1.5×10^5 Pa).

The second factorial design consisted of three variables: particle size in three levels (PS = 0.833, 0.589; 0.417, 0.295, 0.248, and 0.175, 0.124, 0.088 mm), extraction temperature in two levels (T = 20 and 40 °C), and extraction time in three levels (ET = 1, 3, and 6 h).

Factorial experimental designs were used to assess the effects of the operating conditions on the steam distillation and extraction with volatile solvents yields. This experimental planning, at different levels, was made using the SAS statistical package.

Moisture. The humidity of the turmeric rhizomes was determined periodically using Jacobs's method (*19*). The determinations were done in duplicate. The humidity of the dried material was 12% and did not vary during storage.

Characterization and Quantification of Essential Oil. The chemical composition of turmeric essential oil was determined using a model 3300 gas chromatograph (Varian) equipped with a 30 m × 0.25 mm i.d. × 0.25 μm, DB-5 capillary column. Temperature was held at 50 °C for 5 min and raised to 280 °C at 4 °C/min. The injector temperature was 240 °C, and that of the detector was 230 °C. The carrier gas was hydrogen (1.7 mL/min), and 1 μL of sample was injected. The samples were diluted using ethyl acetate: ≅0.005 g of extract in 1 mL of solvent. The split/splitless technique (1:20) was used, and duplicate samples were injected.

To identify the sesquiterpenes quantified in the CG-FID system, samples were also analyzed using a GC-MS system (Shimadzu, QP-5000, Kyoto, Japan), equipped with a 30 m × 0.25 mm i.d. × 0.25 μm, DB-5 fused silica capillary column. The electron impact technique (70 eV) was used. The carrier gas was helium (1.7 mL/min), and 1 μL of sample was injected. The injector temperature was 240 °C, and that of the detector was 230 °C. The temperature programming was 50 °C for 5 min, inversed to 180 °C at 4 °C/min and to 280 °C at 15 °C/min, and held at 280 °C for 19 min. Identification of the chemical constituents was based on (i) comparison of substance mass spectra with the GC-MS system data bank (NIST 62 library), (ii) comparison of mass spectra with data in the literature (*20*−*22*), and (iii) retention indices.

The contents of *ar*-turmerone and (α and β)-turmerone in the extract were calculated using the area percentage obtained from gas chromatography analysis. The three bigger sesquiterpenes found in the turmeric essential oil were *ar*-turmerone, α-turmerone, and β-turmerone, the structures of which are shown in **Figure 2**.

Characterization and Quantification of Pigments. The qualitative determination of pigment amount was done with a Shimadzu spectrophotometer, in the visible range at 425 nm. Pigment concentrations were calculated by the standard curve, and the results, reported in grams of pigments, expressed as curcuminoids. Concentrations in the calibration curve ranged from 9.90×10^{-4} to 6.54×10^{-3} g/L.

A model CG-480C, with a UV−vis 970 detector (Jasco) high-performance liquid chromatograph (HPLC) was used for quantitative analysis of the three curcuminoids, contained in the standards and in the samples. The 220 × 4.6 mm i.d., 5 μm, Spheri-5 amino column (Perkin-Elmer) was used at a flow rate of 1 mL/min. The mobile phase

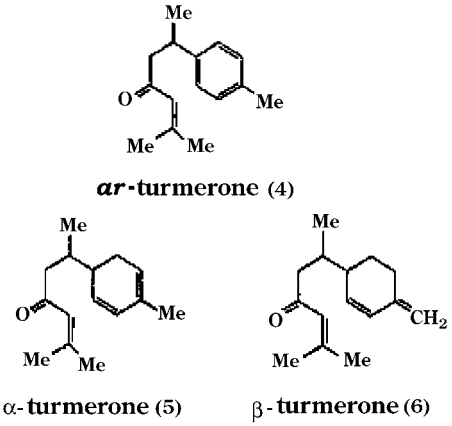

Figure 2. Structures of (**4**) *ar*-turmerone, (**5**) α-turmerone, and (**6**) *β*-turmerone.

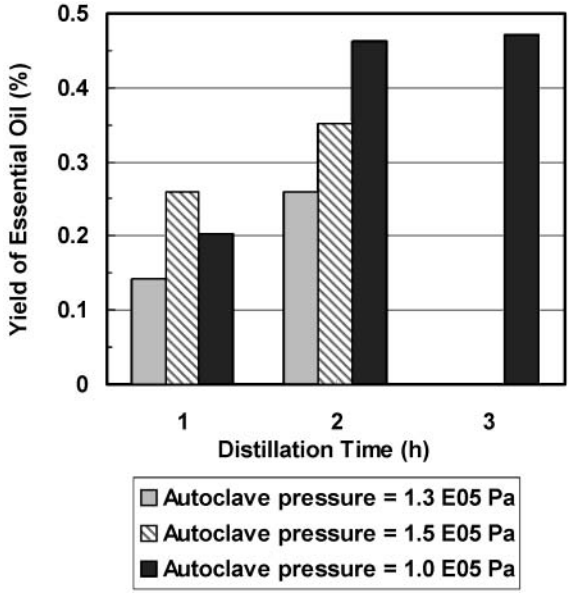

Figure 3. Total yield of essential oil as a function of extraction time and autoclave pressure.

used was ethanol/water (96:4, v/v), the wavelength, $\lambda_{max} = 280$ nm, and the sample volume injected, 20 μL. Concentrations of standard samples varied from 1.50×10^{-2} to 2.0×10^{-2} g/L.

RESULTS AND DISCUSSION

Steam Distillation. Figure 3 shows the operating conditions and the total essential oil yields obtained by steam distillation.

The statistical analysis showed that the process variables time ($p = 0.0962$) and autoclave pressure ($p = 0.2975$) did not have significant effects on the yield. The same was true for the interaction between distillation time and autoclave pressure.

Because the gelatinization of starch takes place above 60 °C (*23*) and the temperatures inside the distiller, at pressures 1.0×10^5, 1.3×10^5, and 1.5×10^5 Pa, were 101, 107, and 110 °C, respectively, the starch contained in the rhizomes gelatinized, which made the yield diminish with an increase in pressure. There was also loss of volatile material, during distillation, due to high temperature.

Total solids were collected at once, at the end of the extraction, together with drain water. After being decanted and dried, 26.78, 0.39, and 1.29 g were obtained at pressures of 1.0×10^5, 1.3×10^5, and 1.5×10^5 Pa, respectively. Despite the difference between solid mass values, the percentage in curcuminoids was ~0.16%, expressed as curcumin, demethoxycurcumin, and bisdemethoxycurcumin. This occurred due to the low solubility of the pigment in hot water.

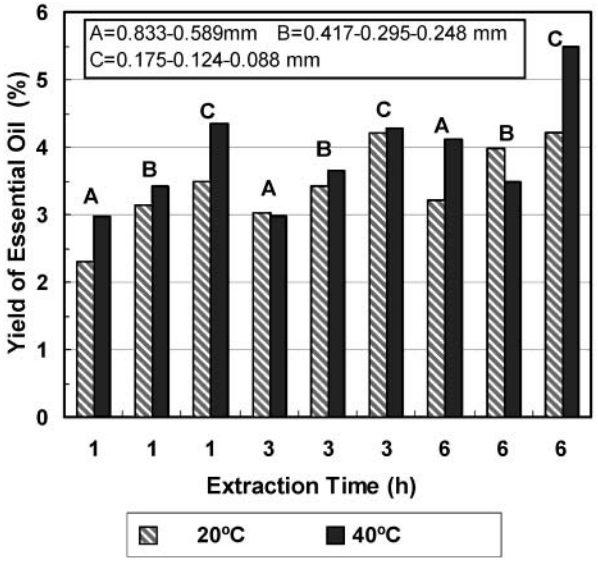

Figure 4. Total yield of essential oil as a function of extraction time, temperature, and particle size.

Extraction with Volatile Solvents. Figure 4 presents the operating conditions along with the total yield of essential oil for the extraction with petroleum ether. The statistical analysis (SAS) showed that particle size (PS), temperature (*T*), and extraction time (ET) significantly affected the yield of extraction ($p = 0.0002$, $p = 0.0253$, and $p = 0.0065$, respectively). However, the interactions between PS, *T*, and ET were not significant. Statistical analysis of the results described in **Figure 4** showed that particle size was the most significant parameter, followed by extraction time and temperature. Therefore, by this parameter analysis, it could be verified that yield increased, at the two temperatures analyzed (20 and 40 °C), as particle size decreased, except for tests 8 (ET = 6 h; PS = 0.417, 0.295, 0.248 mm; $T = 40$ °C) and 4 (ET = 3 h; PS = 0.833, 0.589 mm; $T = 40$°C), probably due to experimental errors. The temperature of 40 °C also favored the increase in yield (average of 12.2%). Therefore, a higher yield was obtained when 0.175, 0.124, 0.088 mm particles were used, at 40 °C with 6 h of extraction (test 9). It can be noted that for smaller particles, extraction time decreased. Therefore, it can be concluded that the extraction could be done in 1 h, diminishing extraction time by 5 h and allowing an amount of essential oil 25% lower to be obtained.

Figure 5 shows the operating conditions and the total pigment yields obtained by extraction with ethanol. A statistical analysis was done using the pigment yield as the response variable. The results showed that the process variables particle size (PS), temperature (*T*), and extraction time (ET) exerted significant effects on the yield ($p = 0.0002$, $p = 0.0142$, $p = 0.0101$, respectively). The yield was also affected by the interaction between PS and *T* ($p = 0.0255$), whereas the interactions of ET with two variables were not significant ($p = 0.9833$ and $p = 0.2159$).

Statistical analysis of considered variables for pigment extraction presented a behavior similar to that of the statistical analysis of the essential oil. Pigment yield increased as particle size diminished, and raising the temperature from 30 to 60 °C increased the yield by 6.8% (average). Therefore, pigment extraction can be performed in 1 h, with the smaller particles, and at 60 °C, allowing yield to be only 7% lower.

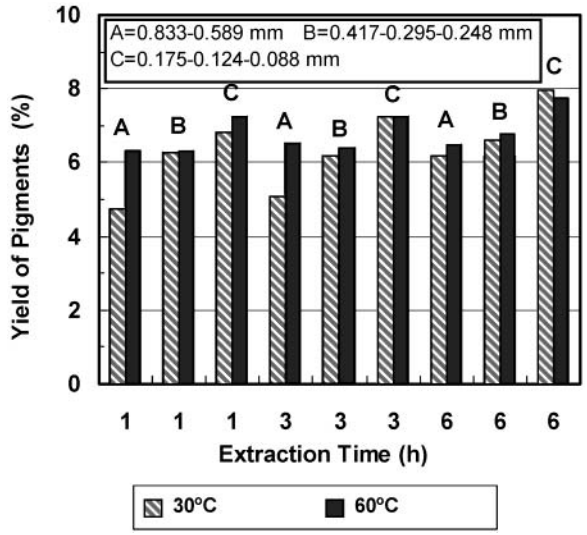

Figure 5. Total yield of pigments as a function of extraction time, temperature, and particle size.

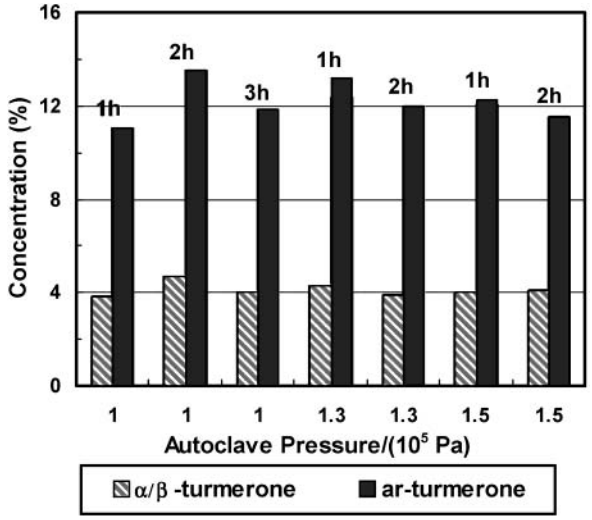

Figure 6. Concentration (percent) of *ar*-turmerone and (α and β)-turmerone as a function of autoclave pressure.

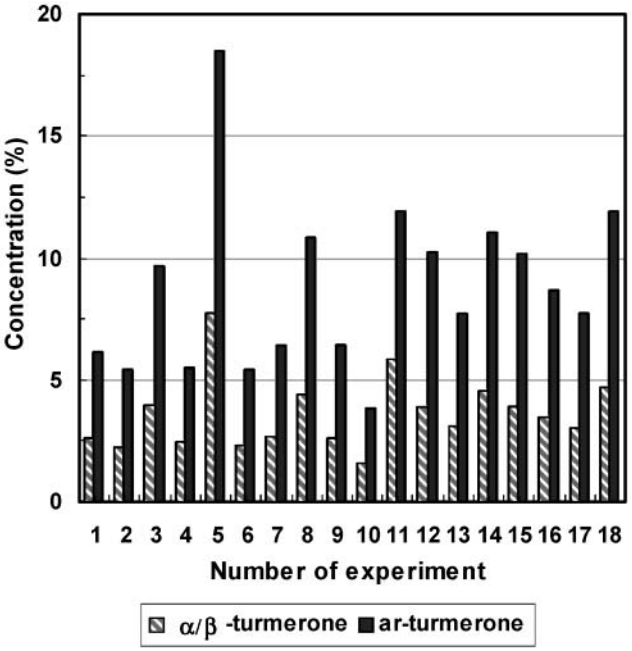

Figure 7. Concentration (percent) of *ar*-turmerone and (α and β)-turmerone as a function of number of experiments.

The essential oil yield was smaller for the steam distillation process as compared to the solvent extraction. Nevertheless, total operational times involved in each process were quite different. The steam distillation required 2 h, whereas 6 h was necessary for the solvent extraction. Other differences between the processes were the color and the odor of the extract. The micelle was dark yellow, whereas the essential oil was clear yellow. Despite that, the micelle odor was closer to the characteristic aroma than was the odor of the essential oil.

Even after the rhizomes had been carefully prepared by proper comminution, only part of the essential oil is present on the surfaces of the material and immediately available for vaporization by steam. The remainder of the oil arrives at the surface only after diffusing through at least a thin layer of plant tissue. Rhizomes can best be handled in a hay or similar device. This action simply reduces the long parts of the plant to short lengths, which are more readily handled in the distillation proper and, above all, ensures a more uniform and compact charge in the still. Otherwise, the live steam would find ready passages through the wide interspaces of uncut rhizomes and escape without coming in close contact with all of the plant particles, but the result, especially in the case of steam distillation, would be a very inferior yield of oil than in the case solvent extraction.

The effects accompanying hydrodistillation are the diffusion of essential oils and hot water through the plant membranes, whence the term hydrodiffusion, and decomposition occasioned by heat. The hydrodiffusion is always a slow process, and if the plants or some of plants were left intact, the rate of recovery of oil would be determined entirely by the rate of diffusion. The term diffusion implies the mutual penetration of different substances until equilibrium was established within the system. The distillation of rhizomes is connected with processes of diffusion and, principally, of osmosis. Distillation offers better conditions for the osmosis of oil, because the higher temperature and the movement of water caused by temperature and pressure fluctuations within the still accelerate the forces of diffusion to such a point that all of the volatile oil contained within the plant tissue can be collected. The pressure of distillation (atmospheric) can be selected, but the temperature of the vapor mixture rising through the charge in the still varies and fluctuates in the course of the operation. It is lowest at the beginning because the lowest boiling constituents of the volatile substances, freed by comminution of the rhizome, vaporize first. To obtain the best quality of oil, it is necessary to ensure that during distillation the essential oil is maintained at low temperature or, at worst, that it be kept at a high temperature for as short a time as possible.

Characterization of the Turmeric Essential Oil and Pigment. Samples of extracts and pigments for various experiments were analyzed by GC-FID and HPLC. The selected samples extracts were also analyzed by GC-MS. **Figures 6** and **7** show the major substances found in the turmeric essential oil and the pigments (**Tables 1** and **2**). The chemical profiles for the essential oil and pigments obtained in the various process conditions was similar. Using this information and the total mass of the essential oil, the relative contents of *ar*-turmerone and (α and β)-turmerone were estimated.

Isomers α-turmerone and β-turmerone were quantified as a single component because it was not possible to separate them by using a 30 m chromatographic column. Despite the fact that the yield of essential oil was higher after 2 h of extraction, only

Table 1. Results in Mass (Grams) of Pigments—Steam Distillation

pressure/(10^5 Pa)	curcumin	demethoxy-curcumin	bisdemethoxy-curcumin
1.0	38.77×10^{-3}	0.726×10^{-3}	4.552×10^{-3}
1.3	0.603×10^{-3}	0.00956×10^{-3}	0.0324×10^{-3}
1.5	1.925×10^{-3}	0.0324×10^{-3}	0.0974×10^{-3}

Table 2. Concentration (Grams per Liter) of Pigments—Extraction with Volatile Solvent

run	curcumin	demethoxy-curcumin	bisdemethoxy-curcumin
1	0.0726	0.0057	0.0030
2	0.0657	0.0049	0.0032
3	0.0658	0.0049	0.0037
4	0.3887	0.0361	0.0275
5	0.4367	0.0404	0.0268
6	0.2808	0.0228	0.0204
7	0.2561	0.0212	0.0154
8	0.3048	0.0271	0.0231
9	0.2924	0.0229	0.0185
10	0.2577	0.0212	0.0152
11	0.2326	0.0173	0.0136
12	0.2420	0.0166	0.0161
13	0.2803	0.0206	0.0160
14	0.2028	0.0147	0.0140
15	0.2810	0.0204	0.0208
16	0.2417	0.0184	0.0149
17	0.2656	0.0204	0.0177
18	0.2305	0.0174	0.0191

at the pressure of 1.0×10^5 Pa were higher concentrations of *ar*-turmerone (13.5%) and (α and β)-turmerone (4.7%) obtained. On the other hand, at the pressures of 1.3×10^5 and 1.5×10^5 Pa, the highest yields of *ar*-turmerone (13.2 and 12.3%) and (α and β)-turmerone (4.3 and 4%) were obtained in 1 h of extraction. Because temperature inside the distiller rose due to the increase in the autoclave pressure, these sesquiterpenes were extracted within a shorter period.

Comparison between **Figures 6** and **7** shows that the amounts of *ar*-turmerone and (α and β)-turmerone present in the petroleum ether extract were smaller compared with the amounts of *ar*-turmerone and (α and β)-turmerone present in the essential oil from the steam distillation process. Whereas the steam distillation relative proportions between *ar*-turmerone and (α and β)-turmerone varied from 12.8 and 4.1%, respectively, on average, for the petroleum ether extraction variations were from 8.7 and 3.6%, respectively, on average, although the amount of essential oil in the steam distillation process was small. As can be observed, the relative amounts of essential oil were significantly affected by the particle size, temperature, and extraction time. Despite the fact that test 9 (PS = 0.175, 0.124, 0.088 mm; $T = 40$ °C; ET = 6 h) presented the higher yield in essential oil, the higher amounts of *ar*-turmerone (28.5%) and (α and β)-turmerone (7.7%) were verified in test 5 (PS = 0.417, 0.295, 0.248 mm; $T = 40$ °C; ET = 3 h).

From the previous discussion, considering solely the amounts of *ar*-turmerone and (α and β)-turmerone for the steam distillation process, the best operational condition process was at 1.0×10^5 Pa for a period of 2 h. A similar analysis for the petroleum ether extraction process would lead one to choose 0.175, 0.124, 0.088 mm as the particle size and to run the process at 40 °C for a period of 3 h. It must be remembered that the petroleum ether extraction process produced an extract poor in *ar*-turmerone and (α and β)-turmerone as compared to the steam distillation process.

Table 1 shows the mass quantity of pigments extracted from drain water by the steam distillation process. Pigments (curcuminoids) were collected only at 2 h time, in each extraction. Chromatographic analysis showed that, among the three curcuminoids obtained, curcumin presented a higher amount in all extractions. However, a rough determination of these pigments dissolved in the decantation water can be accomplished by direct spectrophotometric analysis and showed values of 2.59×10^{-3}, 0.52×10^{-3}, and 1.37×10^{-3} g for runs made at pressures of 1.0×10^5, 1.3×10^5, and 1.5×10^5 Pa, respectively.

Table 2 shows that test 5 (PS = 0.417, 0.295, 0.248 mm; $T = 40$ °C; ET = 3 h) also gave higher concentrations of curcumin (0.4347 g/L), demethoxycurcumin (0.0404 g/L), and bisdemethoxycurcumin (0.0268 g/L), although the yield in essential oil was not the highest. Therefore, it can be concluded that the conditions of test 5 (PS = 0.417, 0.295, 0.248 mm; $T = 40$ °C; ET = 3 h), regarding both the obtaining of curcuminoids and the extraction of the two main substances of the essential oil, were the best conditions to reach a higher fractionation of these components (that is, 18.5% of *ar*-turmerone and 7.7% of α/β-turmerone).

Comparison of the advantages and disadvantages of each process yields the followinglist: Steam distillation requires low-cost equipment. In the case of steam distillation, the temperature is determined entirely by the operating pressure, and higher temperatures usually will increase the rate of diffusion. The solubility of essential oils in water also increases with higher temperatures. Hence, observance of the following principles leads to the best yields and to a high quality of essential oil: (i) maintenance of as low a temperature as is feasible, not forgetting, however, that the rate of production will be determined by the temperature; (ii) in the case of steam distillation, use of as little vapor as possible in direct contact with rhizomes, but keeping in mind that some water should be present to promote diffusion; (iii) thorough comminution of rhizomes before distillation and very careful, uniform packing of the still charge, remembering that in all but steam distillation excessive comminution will result in channeling of steam through the mass of rhizomes, thus reducing efficiency because of poor contact between steam and charge. Conversely, the extraction process can be performed at relatively low temperatures, although to get the final product another step is required to eliminate the solvent, and the yields are higher, but some fractionation of material will certainly be required to separate the essential oil from the pigments, starch, and other contaminants.

From the above, it is easily concluded that the total yield cannot be a single criterion used to select a process and its corresponding conditions. Other variables must be known before a sound decision can be made. Criteria should include both the content of the target substances *ar*-turmerone and (α and β)-turmerone and the content of the pigments in the final product. Therefore, the characterization of the extracts is ultimately very important information. Another question to be considered is that even though solvent extraction increases the yield, this process requires a further purification step, which would include the removal of solvent from both the extract and the solid residue in order to get a purified product and byproduct. Therefore, all of this must be taken under consideration to make a sound decision on the process best suited to produce turmeric of essential oil and pigments for each specific application.

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