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# Valorization of Brazilian Vetiver (*Vetiveria zizanioides* (L.) Nash ex Small) Oil

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The valorization of extracts from Brazilian vetiver (*Vetiveria zizanioides* (L.) Nash ex Small) roots was studied. This study took into account the extraction method, the chemical composition of the extracts, their sensorial characteristics, and the possibility of chemical transformations of the product. The performed extraction methods were hydrodistillation and extraction with supercritical carbon dioxide. Some pretreatment methods were tested on the vetiver roots and evaluated in terms of extraction yield, process time, chemical composition, and sensorial properties. Supercritical carbon dioxide extraction resulted in high yield (3.2%) in significantly less time than the other methods. The chemical compositions of the extracts obtained by the different methods were also compared to those of commercial vetiver oils from other sources, showing that Brazilian samples had a greater acid amount. An extraction in basic medium from Brazilian vetiver oil was done to remove its main acid (zizanoic acid), which was chemically transformed into an alcohol (khusimol) of desirable sensorial properties. Sensory evaluation indicated that the Brazilian volatile oil without acid could be used in perfumery and the extract obtained with supercritical carbon dioxide could have application in food.

**KEYWORDS: Chemical valorization; volatile oil; supercritical carbon dioxide extraction; *Vetiveria zizanioides* (L.) Nash ex Small; vetiver**

## INTRODUCTION

The recovery of volatile oils from vegetal raw materials is an activity of great interest to the industry for the manufacturing of natural products for many purposes. The compounds from volatile oils can have, for example, sensorial properties that make them valorous for the manufacturing of perfumes, other cosmetic products, and foods. Vetiver (*Vetiveria zizanioides* (L.) Nash ex Small) is a plant of the *Gramineae* family widely spread in tropical regions of Asia, Africa, Oceania, and Central and South America. The volatile oil from vetiver roots is a viscous liquid, whose color varies from amber to dark brown, and whose odor has sweet, earthy, and woody notes (*1*). This oil is much appreciated by the perfume industry, where it is used as a fixative and as a odor contributor in bases, such as rose (*2*), chypre (*3*), and in several masculine fragrances. Furthermore, vetiver can be used in the prevention of soil erosion by rain (*4*) and in the combat against soil contamination by heavy metals (*5*), and its oil can be applied in aromatherapy (*6*) and in food, as aroma in canned asparagus and peas (*7*) and as flavor agent in some beverages (*8*).

The main vetiver oil producers are Haiti, Indonesia, and the Reunion Island, and the yearly production is estimated as 140 tons (*9*). Traditionally, the most appreciated vetiver varieties are Haiti and Bourbon, while that of Java, having the weaker organoleptic properties, is the most produced. Bourbon vetiver, which was virtually absent from the market in 1998−1999, has made a modest comeback since then with some 500 kg in 2002 and 2003 (*10*). Finally, the politic instability in Haiti makes the supply of vetiver from this country difficult and irregular (*11*). In Brazil, the vetiver oil production is still low, because of a great variability of the product price and quality.

Steam distillation is the conventional process to obtain vetiver oil. However, the contact between steam and raw material in this process may not be enough to extract all the oil if the roots are not previously treated. The application of previous treatments to the vegetable raw materials, to improve the extraction process, has been tested. Among these pretreatments, the use of enzymatic treatment might open interesting perspectives. For example, this technique was tested on soybean (*12*), sunflower

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*(13)*, and even on lemongrass and lemon eucalyptus (*14*). As in every vegetal organism, the vetiver root cells are protected by a thick wall composed of cellulose and pectin, which may be an obstacle to the extraction. The use of enzymes to act over the cell walls has been tested as a previous step of the oil extraction.

The use of supercritical fluid extraction (SFE) to obtain vegetable extracts can be of great interest to improve the quality of the product, since it is a clean technology, which does not employ organic solvents that may be toxic. Another potential advantage of SFE is that high-process temperatures are not required as in steam distillation, so that the thermal modifications in the chemical composition of the extract may be avoided. SFE has been studied as an extraction method of many products from vegetable sources, which are of interest in food and pharmaceutical industries, like ginger (*15*). Carbon dioxide is an appropriate solvent to these kinds of products because of its low viscosity if compared to liquids and high solubility power compared to gases at near critical or supercritical conditions.

The objective of this work was to evaluate different methods of oil extraction from Brazilian vetiver roots. This evaluation took in account the extraction yields and the quality of the products. The performed extraction methods were hydrodistillation with or without pretreatment and SFE. The extracts obtained with each method from roots collected in Brazil as well as commercial vetiver oils from different sources were chemically analyzed and the chemical composition was related to their sensorial properties. Some chemical reactions were also performed on the acid fraction isolated from the Brazilian vetiver oil, as a trial to improve its quality and acceptability to the market.

## MATERIALS AND METHODS

**Materials.** Vetiver roots were purchased from a local producer in the state of São Paulo, Southeastern Brazil. Some commercial samples of vetiver oil were studied for comparison purposes: volatile oil of vetiver variety Haiti (Charabot, Lot 0000079172, Grasse, France), volatile oil of vetiver variety Java (Charabot, Lot 0015430002, Grasse, France), volatile oil of vetiver variety Bourbon (personal sample), and volatile oil of Brazilian vetiver from the same supplier of the roots.

**Preparation of Raw Material.** Vetiver roots were dried in an air-conditioned room for 48 h and milled in a knife mill (Tecnal TE−631, Piracicaba, Brazil) before every performed extraction procedure. The particles of milled roots were separated according to their particle size in a vibratory sieve system (Series Tyler, W. S. Tyler, Wheeling, IL). The particles with mean diameter of $8.6 \times 10^{-4}$ m were selected for the extraction procedures and stored in a domestic freezer at −18 °C. In some cases, before the hydrodistillation process itself, the vetiver roots were submitted to some pretreatments, with the objective of improving the contact between the oil and the extraction medium. The process from pretreatments to hydrodistillation and SFE is shown schematically in **Figure 1**. The performed pretreatments before hydrodistillation were the following:

*Milling in Liquid Nitrogen.* The roots were cryomilled in liquid nitrogen with a Dangoumill 300 freezer-mill (Prolabo, Paris, France) for 3 min (top impact frequency). The milled roots were stored at −20 °C.

*Treatment with Sodium Hydroxide.* Fifty grams of vetiver roots were immerged into 1 L of a 1 M NaOH solution. The mixture was first kept 8 h under stirring. After that, it was kept without stirring for another 16 h. Then, it was re-acidified with acetic acid until its original pH, which was 4.5, before starting the distillation process.

*Enzymatic Treatment.* For the enzymatic treatment of vetiver roots, two commercial enzymes were used: Celluclast (Lot CCN 3017, Novo-Nordisk, Denmark) and Pectinex Ultra SP-L (Lot KRN 05401, Dittingen, Switzerland). Twenty microliters of each enzyme solution was dissolved into 1 L of an acetic acid solution of pH 4.5, which is the optimal condition for these enzymes (*12*). Fifty grams of vetiver roots were mixed into the solution. The system was shaken for 8 h at room temperature and was kept without shaking for another 16 h before the distillation.

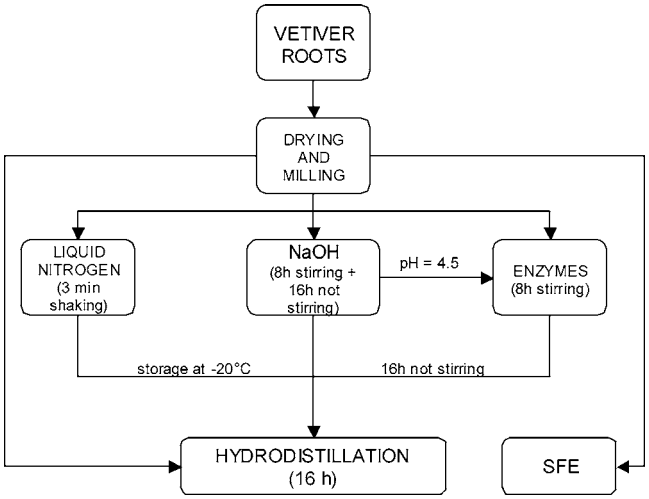


**Figure 1.** Flow chart of the pretreatments of vetiver roots before hydrodistillation.

*Combined Treatment.* A combined pretreatment of NaOH and enzymes was also performed: the roots were treated with NaOH as described previously; after 8 h under stirring and 16 h without stirring, the solution was re-acidified with acetic acid until pH 4.5. Then, the enzymatic treatment was performed as already described.

**Extraction Procedures.** *Hydrodistillation.* A 50-g fraction of vetiver roots was hydrodistilled using a Clevenger type apparatus. The roots were put into a 2-L flask with 1 L of distilled water. Two milliliters of hexane were introduced in the decantation part of the Clevenger apparatus to dissolve the volatile oil during the 16 h of the extraction process. The oil phase was periodically collected and the hexane was evaporated so that the amount of oil could be evaluated in function of the distillation time.

*Supercritical Fluid Extraction (SFE).* An experimental design of two factors (temperature and pressure) and two levels (30 and 40 °C, 80 and 200 bar) was performed to define the conditions in which the highest yield in extract was achieved. Because the extraction was exhaustive for these experiments, the extraction time used was 2 h. The conditions that provided the highest yield were 40 °C and 200 bar, so this temperature and pressure were used for all SFE experiments. Thirty grams of vetiver roots were used in each SFE experiment, with a $CO_2$ flow rate of $6.9 \times 10^{-5}$ kg/s, during 1 h. The SFE unit used was built in the Technical University of Hamburg-Harburg (TUHH, Hamburg, Germany) (*16*). The $CO_2$ at the required conditions was pumped into the line, entering in contact with the vetiver roots compacted in a 100-mL stainless steel column. The mixture of $CO_2$ and extract had its pressure decreased in the outlet valve, so the extract could be collected in a flask cooled with ethylene glycol and then weighed. A micrometer valve was used to control the $CO_2$ flow rate that was measured in a calibrated rotameter. The extraction yield was measured as the ratio between mass of extract and dried raw material.

The extract was separated from the solvent through expansion in the micrometer valve by reducing the pressure to ambient. Some samples of SFE extract were submitted to a hydrodistillation process at the conditions previously described to get the ratio between volatile and nonvolatile components and to determine the chemical composition of the volatile fraction.

**Chemical Composition of Vetiver Oil.** The extracted samples from Brazilian vetiver roots, as well as commercial oil samples from varieties Haiti, Java, Bourbon, and Brazil were analyzed by gas chromatography (GC) and GC-mass spectrometry.

*Gas Chromatography.* GC analyses were performed on a Varian gas chromatograph, model CP-3380, equipped with flame ionization detector containing two silica capillary columns: CP Sil 5 CB low bleed/MS (100% dimethyl polysiloxane, Chrompack/Varian, Palo Alto,

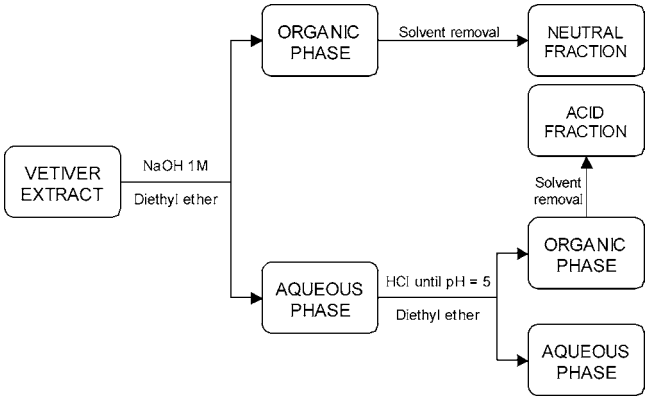


**Figure 2.** Flow chart of the separation of acid and neutral fractions of vetiver oil.

CA) capillary column (30 m × 0.25 mm i.d. × 0.25 μm film) and Supelcowax 10 (poly(ethylene glycol), Supelco Inc, Bellefonte, PA) fused capillary column (30 m × 0.25 mm i.d. × 0.25 μm film); $N_2$ was the carrier gas at 0.8 mL/min; injection type (1.0 μL of sample dissolved in 1 mL of diethyl ether) split ratio, 1:50; injector temperature, 220 °C; detector temperature, 250 °C; temperature program 50−200 °C at 5 °C/min. The linear retention indices of the components were determined relative to the retention times of a series of *n*-alkanes and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors.

*Gas Chromatography−Mass Spectrometry*. GC/MS analyses were performed using an Agilent 6890 GC equipped with a DB-Wax fused silica column (30 m × 0.25 mm i.d. × 0.25 μm) and interfaced with an Agilent mass selective detector (Model 5973 Network, Palo Alto, CA); temperature-programmed at 40−220 °C (3 °C/min); injector temperature, 245 °C; carrier gas, helium; flow rate, 1.1 mL/min; injection type, split (1 μL of sample dissolved in 1 mL of diethyl ether); ionization voltage, 70 eV; electronmultiplier, 1400 eV; mass range [35−300]; scan rate, 2.96 scan/s. A Hewlett-Packard GC 5890A was also used, equipped with an HP1 (cross-linked methyl siloxane, Hewlett-Packard, Wilmington, DE) fused silica column (30 m × 0.25 mm i.d. × 0.25 μm) and interfaced with a quadrupole detector (Model 5970, Palo Alto, CA). Column temperature was programmed from 70 to 200 °C at 10 °C/min; injector temperature was 220 °C. Helium was used as carrier gas at a flow of 0.6 mL/min; the mass spectrometer operated at 70 eV.

The identification of the constituents was assigned on the basis of comparison of retention data and mass spectra with those of the data bank NBS75K (*17*), literature data (*18*−*24*), and stored laboratory mass spectral library.

**Chemical Valorization of Vetiver Oil.** The process of chemical valorization of vetiver oil consisted in the development of a method to improve the sensorial properties of the extracts. This process had two steps: first, the separation of the acid fraction from the oil, which has poor sensorial properties; second, the chemical transformation of the acid into khusimol, which may be a valuable byproduct of the vetiver oil industry.

*Removal of Acids from Vetiver Oil*. The ionizable fraction of the vetiver oil was isolated by extraction with NaOH and diethyl ether. Fifteen milliliters of a 0.2 g/mL ethereal solution of the vetiver oil (Brazilian commercial sample) was extracted with 40 mL of 1 M NaOH aq, and the extract was acidified with HCl 2 N until pH near 5. The liberated acids were then extracted by 40 mL of diethyl ether. The ethereal solutions of the acid and neutral fractions of vetiver oil were washed with water and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. Both acid and neutral fractions were analyzed by GC. **Figure 2** shows a flowchart of the acid removal process from vetiver oil.

The main component of the acid fraction, zizanoic acid, was purified by flash chromatography on silica gel 60 column (Merck, 70−230 mesh ASM) with petroleum ether/ethyl acetate as eluent. Its specific optical rotation was measured at 25 °C by using a polarimeter Perkin-Elmer 241 as $[\alpha]_D = 26.5°$ (*c* 1.14 in EtOH).

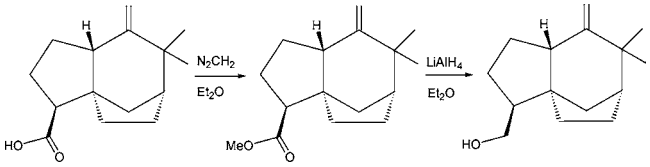


**Figure 3.** Synthesis of khusimol from zizanoic acid.

**Table 1.** Yields and Times of Extraction from Brazilian Vetiver Roots with Different Methods

| method | extraction time (h) | yield (%) |
|---|---|---|
| hydrodistillation (HD) | 16 | 1.8 ± 0.1 |
| HD–$N_2$ | 16 | 1.8 ± 0.1 |
| HD–NaOH | 16 | 1.8 ± 0.1 |
| HD–enzymes | 16 | 1.9 ± 0.1 |
| HD–NaOH and enzymes | 16 | 1.7 ± 0.1 |
| SFE (200 bar, 40 °C) | 1 | 3.2 ± 0.2 |
| HD from SFE extract | 12 | 0.6 ± 0.1 |

*Synthesis of Khusimol from Zizanoic Acid*. The zizanoic acid obtained by extraction in basic medium was esterified with diazomethane ($N_2$-$CH_2$). The resulting ester (methyl zizanoate) was purified on a silica gel 60 column and reduced to khusimol with $LiAlH_4$ (*25*). **Figure 3** shows the steps of the chemical transformation of zizanoic acid to khusimol. All the steps of this transformation were analyzed by thin-layer chromatography (TLC), using petroleum ether:ethyl acetate (8:2) as eluent, on a silica plate. The TLCs were revealed with bromocresol blue for the first step and potassium permanganate for the second. The intermediary and final products were also analyzed by GC and GC-MS.

**Sensory Evaluation.** The commercial oil samples from Brazil, its acid and nonionizable fractions, and the extracts obtained by SFE were evaluated by perfumery specialists (Societé Ayrel, Liège Belgium and Société IPRA, Grasse, France). They took into account the typical odor profiling desired in vetiver oil for perfumery applications. The test consisted in an olfactory description of each sample.

## RESULTS AND DISCUSSION

**Extraction. Table 1** shows the extraction yields (w/w) obtained with each method of extraction, in terms of mass of vetiver roots in dry basis. As can be observed, the pretreatments of the vetiver roots before hydrodistillation did not significantly increase the yield and rate of extraction. Higher extraction yield was obtained by extraction with supercritical carbon dioxide, which can be explained by the presence of larger amounts of nonvolatile compounds, such as fatty acids and waxes. The yield obtained by hydrodistillation of the SFE extract was 18.8%, in terms of extract, after 12 h of process. This result indicates that the low yields observed for the hydrodistillation process were not related to the structure of the vetiver plant and also that indeed the SFE process produced extracts containing heavier molecular mass compounds. The SFE process was much faster than the other extraction techniques, since 1 h was enough to achieve yields higher than those obtained after 16 h of hydrodistillation. In terms of volatile oil, hydrodistillation resulted in higher yields. However, compounds of interest for other applications may be obtained only with SFE, in the case we need to avoid organic solvents.

**Extracts.** The commercial oil samples from Brazil, and the varieties Haiti, Java, and Bourbon, are viscous dark brown liquids, which agree with the literature description (*26*). The oils obtained by hydrodistillation had a similar viscosity, but their colors varied from yellow to light brown. This difference in colors may be due to the high temperatures used in steam distillation of commercial vetiver oil. The SFE extract was light-yellow colored and quite more viscous than the commercial samples.

**Table 2.** Chemical Composition of the Volatile Fraction of Vetiver Extracts (Percent of Total)

| compound | *a* | *b* | *c* | *d* | *e* | *f* | *g* | *h* | RI 1[i] | RI 2[i] | ref |
|---|---|---|---|---|---|---|---|---|---|---|---|
| α-ylangene | | | 0.1 | | 0.1 | | | | 1465 | | *18* |
| pre-zizaene | 1.0 | | 0.4 | | 0.6 | 0.4 | 0.4 | 0.8 | 1590 | 1375 | *19* |
| khusimene | 1.7 | 0.5 | 0.7 | 0.7 | 0.5 | 0.9 | | 3.0 | 1620 | 1468 | *18* |
| α-amorphene | 1.6 | 0.3 | 0.5 | 0.4 | 0.4 | 1.8 | 2.1 | 4.2 | 1676 | 1491 | *18* |
| *cis*-eudesma-6,11-diene | 1.2 | | | | | 1.4 | 0.8 | 2.4 | 1692 | 1498 | *18* |
| *cis*-β-guaiene | | 0.2 | 0.5 | 0.3 | 0.8 | | | | 1702 | | *18* |
| δ-amorphene | 1.4 | 0.3 | 0.4 | 0.4 | 0.2 | 1.1 | 1.8 | 3.5 | 1710 | 1519 | *18* |
| β-vetispirene | 1.0 | | 0.1 | | 0.2 | 1.1 | 1.0 | 2.7 | 1737 | 1506 | *20* |
| γ-cadinene | 0.6 | 0.2 | 0.4 | 0.3 | 0.3 | | 0.3 | 0.7 | 1752 | 1531 | *18* |
| γ-vetivenene | 1.3 | | | | | | 0.8 | 5.1 | 1813 | 1540 | *20* |
| β-vetivenene | 2.0 | | | | 0.4 | 1.6 | 1.7 | 5.2 | 1852 | 1574 | *20* |
| α-calacorene | 0.9 | | | | | 0.8 | | 0.7 | 1914 | 1552 | *18* |
| *cis*-eudesm-6-en-11-ol | 1.9 | 1.5 | 2.1 | 1.7 | 1.7 | 2.4 | 2.1 | 1.1 | 2064 | 1575 | *21* |
| khusimone | 3.6 | 2.4 | 4.5 | 2.4 | 2.6 | 3.5 | 3.9 | 2.6 | 2175 | 1616 | *18* |
| ziza-6(13)-en-3-one | 2.5 | 1.8 | 2.9 | 2.0 | 2.0 | 1.4 | 2.8 | 2.1 | 2227 | | *19* |
| khusinol | 3.4 | 1.5 | 2.7 | 2.0 | 2.2 | 1.9 | 1.7 | 2.4 | 2292 | 1699 | *18* |
| khusian-2-ol | 3.4 | 1.6 | 2.7 | 2.2 | 2.4 | 3.4 | 2.8 | 1.3 | 2323 | 1715 | *22* |
| vetiselinenol | 1.7 | 0.8 | 1.8 | 1.1 | 1.3 | 2.3 | 1.8 | 1.0 | 2343 | | *23* |
| cyclocopacamphan-12-ol | 1.0 | 0.6 | 1.0 | 0.9 | 0.8 | 1.7 | 1.3 | 0.3 | 2351 | | *19* |
| 2-*epi*-ziza-6(13)-3α-ol | 1.9 | 1.1 | 2.8 | 1.7 | 1.9 | 1.6 | 1.2 | 1.1 | 2406 | | *22* |
| isovalencenal | 1.6 | 1.8 | 2.5 | 2.9 | 1.5 | 2.5 | 2.1 | 1.0 | 2453 | | *19* |
| β-vetivone | 1.5 | 0.8 | 4.8 | 5.5 | 1.9 | 5.6 | 3.9 | 8.0 | 2519 | 1829 | *18* |
| khusimol | 7.2 | 7.2 | 8.3 | 11.8 | 9.5 | 13.3 | 6.4 | 9.7 | 2521 | 1774 | *18* |
| nootkatone | 1.1 | 1.2 | 1.7 | 2.2 | 1.1 | 0.4 | 0.4 | | 2539 | 1819 | *18*[j] |
| α-vetivone | 5.4 | 5.4 | 10.4 | 6.8 | 4.9 | 4.8 | 3.3 | 4.0 | 2559 | 1851 | *18* |
| isovalencenol | 3.0 | 7.4 | 10.2 | 11.0 | 8.3 | 15.3 | 8.9 | 4.4 | 2567 | 1813 | *24* |
| bicyclovetivenol | 0.5 | 1.2 | 0.6 | 2.4 | 0.2 | 1.1 | 0.8 | | 2604 | | *18* |
| zizanoic acid | 11.8 | 32.4 | 6.7 | 3.0 | 24.0 | 0.5 | 0.9 | 3.3 | >2800 | 1837 | *16* |
| hydrocarbons | 12.7 | 1.5 | 3.1 | 2.1 | 3.5 | 9.1 | 8.9 | 28.3 | | | |
| alcohols | 24.0 | 22.9 | 32.2 | 34.8 | 28.3 | 43.0 | 27.0 | 21.3 | | | |
| carbonyl compounds | 15.7 | 13.4 | 26.8 | 21.8 | 14.0 | 18.2 | 16.4 | 17.7 | | | |
| carboxylic acids | 11.8 | 32.4 | 6.7 | 3.0 | 24.0 | 0.5 | 0.9 | 3.3 | | | |
| total identified | 64.2 | 70.2 | 68.8 | 61.7 | 69.8 | 70.8 | 53.2 | 70.6 | | | |

[a] Brazilian commercial oil. [b] SFE extract. [c] Hydrodistilled SFE extract. [d] Neutral fraction from Brazilian oil. [e] Hydrodistilled oil. [f] Haiti oil. [g] Bourbon oil. [h] Java oil. [i] RI 1, retention index on Carbowax column; RI 2, retention index on DB1 column. [j] Authentic sample. All identifications were tentative.

**Table 3.** Chemical Composition of Vetiver Oils by Group (Percent of Total Identified)[a]

| chemical group | *a* | *b* | *c* | *d* | *e* | *f* | *g* | *h* |
|---|---|---|---|---|---|---|---|---|
| hydrocarbons | 19.8 | 2.1 | 4.5 | 3.4 | 5.4 | 12.9 | 16.7 | 40.1 |
| alcohols | 37.4 | 32.6 | 46.8 | 56.4 | 40.5 | 60.7 | 50.8 | 30.2 |
| carbonyl compounds | 24.5 | 19.1 | 39.0 | 35.3 | 20.1 | 25.7 | 30.8 | 25.1 |
| carboxylic acids | 18.4 | 46.2 | 9.7 | 4.9 | 34.4 | 0.7 | 1.7 | 4.7 |

[a] Brazilian commercial oil. [b] SFE extract. [c] Hydrodistilled SFE extract. [d] Neutral fraction from Brazilian oil. [e] Hydrodistilled oil. [f] Haiti oil. [g] Bourbon oil. [h] Java oil.

**Chemical Composition.** Twenty-eight compounds were identified by GC and GC-MS in the vetiver oils, being exclusively sesquiterpenes. **Table 2** shows the composition of the volatile fractions, that is, the substances that are detectable by GC-FID, of the extracts obtained from Brazilian vetiver roots, by different methods, along with those of vetiver samples from different origins. At the bottom of **Table 2**, the identified components were gathered according to their chemical function. In general, the percentages of the main compounds in the commercial vetiver oils of varieties Haiti, Java, and Bourbon agreed with the range proposed by the international standard (*24*).

The chemical profile of the oils obtained by hydrodistillation was almost the same, independently of the previous treatment applied to the vetiver roots. In **Tables 2** and **3**, the chemical composition of the hydrodistilled oil without any previous treatment is shown.

As can be seen in **Table 2**, all the samples obtained from Brazilian vetiver contained a high amount of zizanoic acid, except the hydrodistilled fraction from the SFE extract, where most of this compound was eliminated, probably because of the shorter distillation time (12 h). However, the final concentration of acid in this sample is still higher than the ones found in the vetiver oils from varieties Haiti, Bourbon, and Java, and

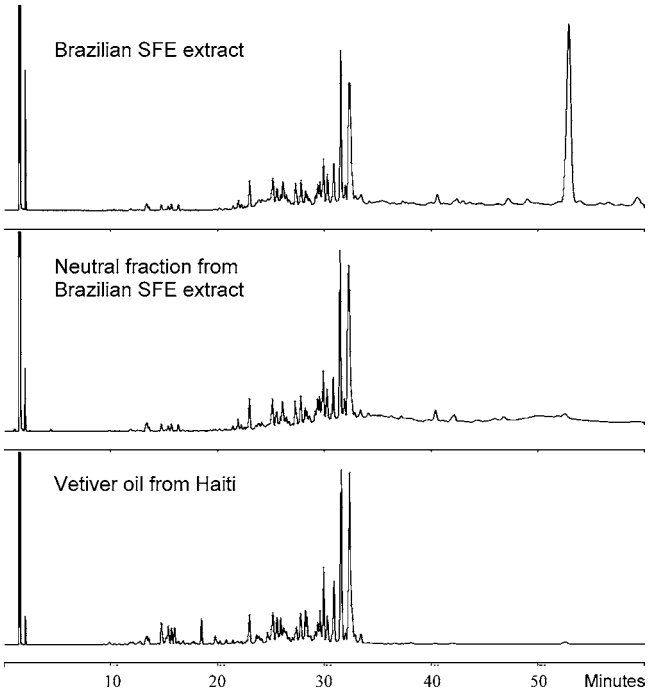


**Figure 4.** Gas chromatograms of vetiver SFE extract, its neutral fraction, and commercial vetiver oil from Haiti.

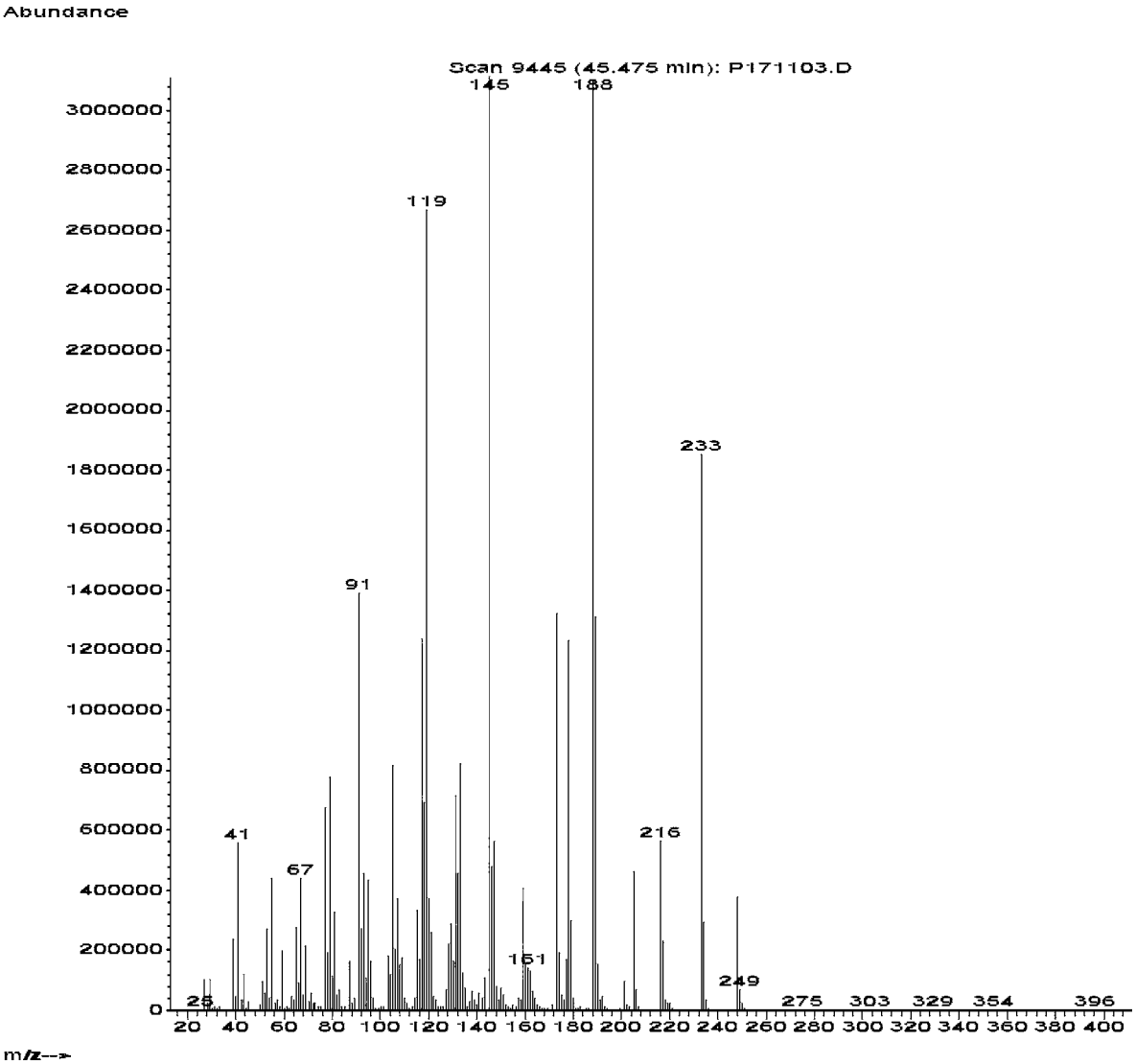


**Figure 5.** Mass spectrum of the product of the esterification of zizanoic acid with diazomethane, identified as methyl zizanoate.

the yield of this extraction was quite lower than those obtained with the other methods.

The amounts of alcohols are higher in the varieties Haiti and Bourbon than in the samples from Brazil and variety Java. This chemical characteristic is directly related to the quality of the volatile oil, since the alcohols (mainly khusimol) are responsible for the desired woody odor of vetiver oil (*1*). The variety Java is quite richer in sesquiterpenic hydrocarbons, while the Brazilian oil presents a high amount of acids, mainly zizanoic acid. Both acids and hydrocarbons are not desired in the composition of vetiver oil for perfumery applications, because of their poor sensorial properties.

**Removal of Acids from Vetiver Oil.** A GC analysis indicated the presence of 76% of zizanoic acid in the volatile ionizable fraction, which represented 23% of the total extract. The specific optical rotation of zizanoic acid after purification by flash chromatography was in agreement with literature data (*25*) that allowed to specify the stereostructure shown in **Figure 3** as well as those of the related products (methyl zizanoate and khusimol) obtained by chemical transformation.

By the other side, the GC analysis of the nonionizable (neutral) fraction showed a profile near to those of the oil samples from vetiver varieties Haiti and Bourbon, richer in sesquiterpenic alcohols, and practically free from zizanoic acid, as can be observed in **Tables 2** and **3** and **Figure 4**. This neutral fraction represented 77% of the total extract. This result suggests that a simple washing of the oil with a basic solution can remove most of the acid compounds from the sample and make its chemical composition similar to those of the highest quality vetiver oils, like the oils from vetiver varieties Haiti and Bourbon.

**Synthesis of Khusimol from Zizanoic Acid.** One of the main compounds identified in the Brazilian vetiver oil was zizanoic acid, which has no sensorial qualities. Nevertheless, this acid could be chemically transformed by esterification and reduction into khusimol, whose woody odor is highly appreciated.

Zizanoic acid was esterified with diazomethane to afford a compound with high purity level (86.3%) and 72% yield, which was confirmed to be methyl zizanoate by comparison of its mass spectrum with literature (*19*). **Figure 5** shows the mass spectrum of this product.

The reduction of methyl zizanoate with $LiAlH_4$ resulted in a quite pure product (91.0%), which was confirmed to be khusimol, by GC-MS (*18*). The reaction yield was 90.7%. **Figure 6** shows the mass spectrum of the reaction product.

The yield of the chemical transformation of zizanoic acid to khusimol, as well as the purity of the final product, indicates that this transformation might be a good alternative to increase

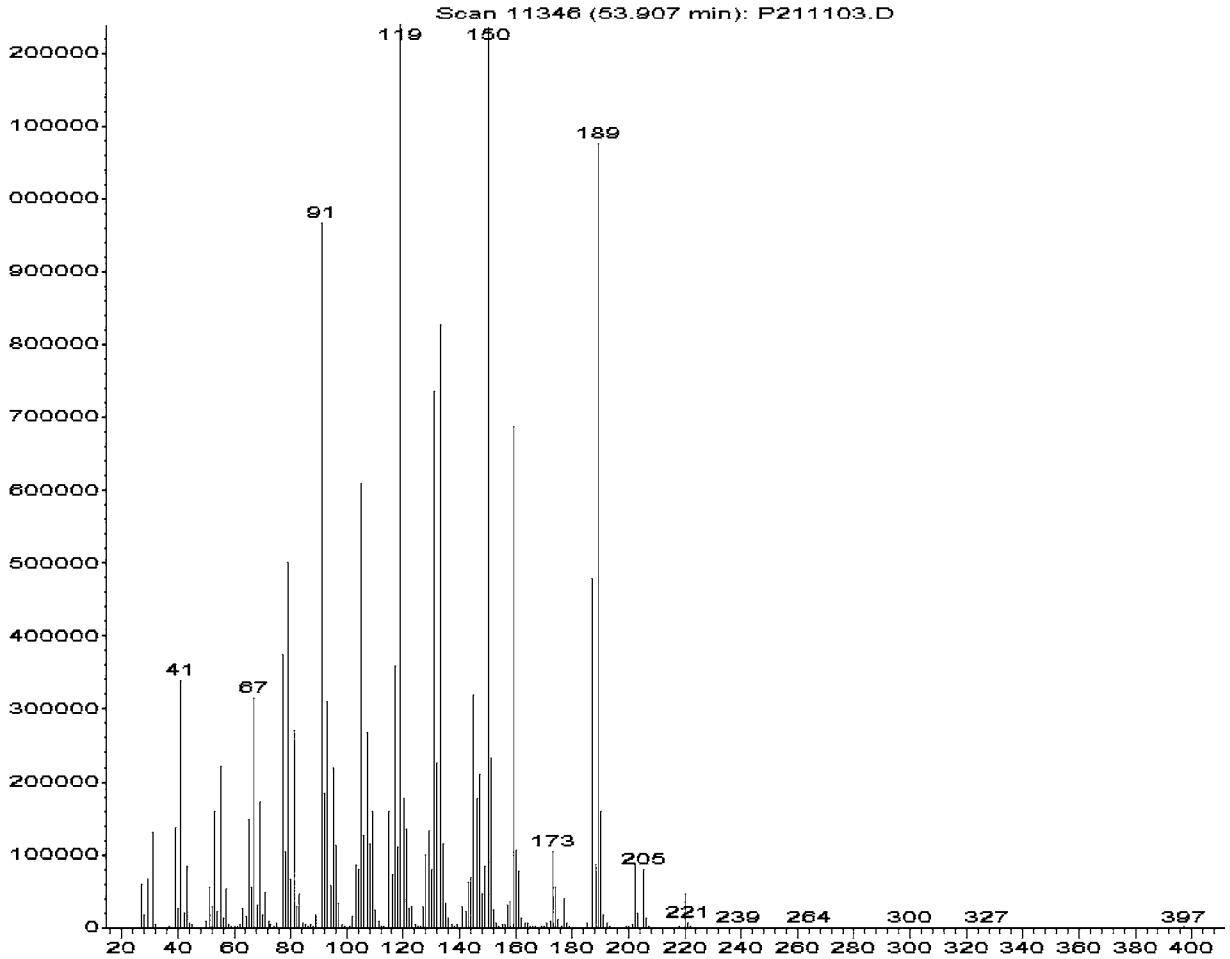


**Figure 6.** Mass spectrum of the product of the reduction of methyl zizanoate with $LiAlH_4$, identified as khusimol.

the alcohol ratio in vetiver oil and make its chemical composition similar to those of commercial samples from vetiver of varieties Haiti and Bourbon (see **Table 3**). Nevertheless, the esterification and reduction process cannot be performed on the whole oil because of the possibility of changing all its chemical composition, even losing desirable compounds. Then, the zizanoic acid should always be separated previously. In industrial scale processes, it is possible then to think about improving the Brazilian essence quality by simply washing it with basic solutions, which would result in the separation of its acid fraction, without using organic solvents. The reduction of the zizanoic acid recuperated by this method in khusimol might then provide an interesting aggregated value product.

The esterification of zizanoic acid with diazomethane is not viable in industrial scale because of the instability of the reagent and the quite dangerous conditions, which make this process viable only in laboratory scale. It would be necessary then to find another method for this transformation, which could be applicable in high scale, such as enzyme esterification under supercritical conditions (*27*). Since this method does not involve toxic or dangerous reactants, the resulting product would preserve its natural character.

**Sensory Evaluation.** The olfactive descriptions of the samples from Brazilian vetiver roots were the following:

*Commercial Sample.* Similar to a vetiver oil type Haiti; woody and earthy without specific character; not green; not warm enough; poorly sweet; without burned note.

*Nonionizable (Neutral) Fraction of the Commercial Sample.* Woody but much less earthy; still less green and warm; could be used like chypre base.

*Acid Fraction of the Commercial Sample.* Slightly balsamic; warm, sweet; not powerful.

*Supercritical Carbon Dioxide Extract.* Without specific vetiver character; with reminiscence of orris resinoid; not powerful; could be used as food aroma for potatoes or asparagus.

These results show that the neutral fraction from vetiver oil has some sensorial properties that are not present in the SFE extract and are interesting for perfumery applications. The removed acid fraction is not applicable in perfumery but might be used for other purposes, as well as the SFE extract. The presence of zizanoic acid in both SFE and commercial samples may be partly responsible for the poor sensorial properties detected. Although a sensorial analysis of the hydrodistilled fraction from the SFE extract was not done, its chemical composition suggests that this sample could be useful for application in perfumes, since its amount of alcohols is close to that found in the Bourbon oil and the acid content is quite low.

Different extraction methods were tested on vetiver roots collected in Brazil. The highest yield was achieved through supercritical fluid extraction, but this extract presented the highest amounts of undesirable compounds, such as zizanoic acid, and other nonvolatile substances. The shortest extraction time was achieved using SFE. The pretreatments of vetiver roots did not influence the yield of hydrodistillation or the composition of the oil. The Brazilian commercial oil and the extracts obtained by hydrodistillation of vetiver roots from the same origin also presented high amounts of zizanoic acid, when compared to commercial samples from other sources. However, this work has shown the possibility of valorizing the Brazilian vetiver oil

by removal, isolation, and chemical transformation of its main acid to the corresponding alcohol.

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## LITERATURE CITED

(1) Arctander, S. *Perfume and Flavor Materials of Natural Origin*; Arctander: Elizabeth, NJ, 1960; 649−653.
(2) Chowdhury, A. R.; Kumar, D.; Lohani, H. GC-MS analysis of essential oils of *Vetiveria zizanioides* (Linn.) Nash., roots. *Fafai J.* **2002**, *April-June*, 33−35.
(3) Weyerstahl, P.; Marschall, H.; Splittgerber, U.; Wolf, D. New sesquiterpene ethers from Vetiver oil. *Liebigs Ann.* **1996**, 1195−1199.
(4) Tscherning, K.; Leihner, D. E.; Hilger, T. H.; Müller-Sämann, K. M.; El-Sharkawy, M. A. Grass barriers in cassava hillside cultivation: Rooting patterns and root growth dynamics. *Field Crops Res.* **1995**, *43*, 131−140.
(5) Chen, H. M.; Zheng, C. R.; Tu, C.; Shen, Z. G. Chemical methods and phytoremediation of soil contaminated with heavy metals. *Chemosphere* **2000**, *43*, 229−234.
(6) Baudoux, D. *L'aromathérapie. Se soigner par les huiles essentielles*; Amyris SPRL: Bruxelles: Belgium, 2002.
(7) Les Gammes de Matières Premières Charabot, accessed on March, 2004, http://www.charabot.fr/MP/mp_gamme.asp
(8) Solomon, C. *Encyclopedia of Asian Food*; Periplus: Boston, MA, 1998.
(9) Chomchalow, N. *Manual of the International Training Course on the Vetiver System*; ORDPB: Bangkok, Thailand, 2000.
(10) Géranium et Vétyver Bourbon: Etat des lieux. *Parfums Cosmétiques Actualités* **2003**, *174*, 42−43.
(11) Le marché des plantes à parfums. *Parfums Cosmétiques Actualités* **1998**, *114*, 68−69.
(12) Dominguez, H.; Nunez, M. J.; Lema, J. M. Enzyme-assisted hexane extraction of soya bean oil. *Food Chem.* **1995**, *54*, 223−231.
(13) Dominguez, H.; Siniero, J.; Nunez, M. J.; Lema, J. M. Enzymatic treatment of sunflower kernels before oil extraction. *Food Res. Int.* **1996**, *6*, 537−545.
(14) Dudai, N.; Weinberg, Z. G.; Larkov, O.; Ravid, U.; Ashbell, G.; Putievsky, E. Changes in essential oil during enzyme-assisted ensiling of Lemongrass (*Cymbopogon citratus* Stapf) and lemon Eucalyptus (*Eucalyptus citriodora* Hook). *J. Agric. Food Chem.* **2001**, *49*, 2262−2266.
(15) Martinez, J.; Monteiro, A. R.; Rosa, P. T. V.; Marques, M. O. M.; Meireles, M. A. A. Multicomponent model to describe extraction of ginger oleoresin with supercritical $CO_2$. *Ind. Eng. Chem. Res.* **2003**, *42*, 1057−1063.
(16) Zetzl, C.; Brunner, G.; Meireles, M. A. A. Standardized low-cost batch SFE−units for university education and comparative research. *Proceedings of the 6th International Symposium on Supercritical Fluids*, Versailles, France, 2003; Vol. 1, pp 577−581.
(17) McLafferty, F. W.; Stauffer, D. B. *The Wiley/NBS Registry of Mass Spectral Data*; Wiley: New York, 1989; Vol. II.
(18) Adams, R. P. *Identification of essential oil components by gas chromatography/quadrupole mass spectrometry*; Allured Publishing: Carol Stream, IL, 2001.
(19) Weyerstahl, P.; Marschall, H.; Splittgerber, U.; Wolf, D. 1,7-Cyclogermacra-1(10),4-dien-15-al, a sesquiterpene with a novel skeleton, and other sesquiterpenes from Haitian vetiver oil. *Flavour Fragrance J.* **2000**, *15*, 61−83.
(20) Joulain, D.; König, W. A. *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*; E. B. Verlag: Hamburg, Germany, 1998.
(21) Weyerstahl, P.; Marschall, H.; Splittgerber, U.; Wolf, D. New *cis*-eudesm-6-ene derivatives from Vetiver oil. *Liebigs Ann/Recueil* **1997**, 1783−1787.
(22) Weyerstahl, P.; Marschall, H.; Splittgerber, U.; Wolf, D. Analysis of the polar fraction of Haitian vetiver oil. *Flavour Fragrance J.* **2000**, *15*, 153−173.
(23) Andersen, N. H. The Structures of Zizanol and Vetiselinenol. *Tetrahedron Lett.* **1970**, *21*, 1755−1758.
(24) Norme Internationale ISO/FDIS 4716:2002 (F) Huile essentielle de vetiver [*Vetiveria zizanioides* (L.) Nash], ISO: 2002.
(25) Hanayama, N.; Kido, F.; Tanaka, R.; Uda, H.; Yoshikoshi, A. Sesquiterpenoids of Vetiver Oil−I: The structures of zizanoic acid and related constituents. *Tetrahedron* **1973**, *29*, 945−954.
(26) Poucher, W. A. *Perfumes, Cosmetics & Soaps*; Chapman and Hall: London, U.K., 1974; Vol. 1.
(27) Goddard, R.; Bosley, J.; Al-Duri, B. Esterification of oleic acid and ethanol in plug flow (packed bed) reactor under supercritical conditions−Investigation of kinetics. *J. Supercrit. Fluids* **2000**, *18*, 121−130.

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