Extraction of Essential Oils and Cuticular Waxes with Compressed CO_2: Effect of Matrix Pretreatment

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The effect of the pretreatment of the matrix on the CO_2 extraction of essential oils and cuticular waxes from aromatic herbs was studied. The vegetable matrix, oregano bracts, was submitted to different mechanical and physical treatments prior to extraction with compressed CO_2 under standard conditions (100 bar, 310 K, and 0.25 kg/h). The rates and yields of extraction of both essential oils and cuticular waxes were evaluated and compared with those obtained from an untreated matrix. The losses of essential oils during the pretreatments were estimated. Comminution under atmospheric and cryogenic conditions and comminution in a closed atmosphere (internal comminution) were the mechanical pretreatments under investigation. The physical disruption of essential oils glands by a fast decompression treatment (FD treatment) was also tested. The structure of the matrixes and essential oils glands, before and after CO_2 extraction, were observed by scanning electron microscopy (SEM). Cryogenic comminution, internal comminution and FD treatment were all found to be effective matrix pretreatments for the extraction of essential oils. However, the selective liberation of the essential oils with respect to cuticular waxes obtained by the FD treatment results in a final extract with a higher content of essential oils. Moreover, the essential oils themselves are believed to be of better quality than those obtained from mechanically treated matrixes.

Introduction

Extraction of essential oils with compressed CO_2 is a modern technique that competes with distillation and organic solvent extraction. Its main advantages include the high quality of the extract (absent of solvent residues and degradation artifacts), the safe and environmentally friendly operation, and in some cases, the lower operational costs of the plant. On the other hand, the high capital cost of the extraction plant, the tendency toward co-extraction of higher-molecular-weight waxy compounds (cuticular waxes),[1−3] and the need for an efficient matrix pretreatment are the major drawbacks of the CO_2 extraction process.

The need for a matrix pretreatment is related to the location of the essential oils within the herbaceous matrixes. Essential oils are produced in glandular cells and stored in the subcuticular space formed at the gland apex.[4,5] These glands develop on the surface of leaves, bracts, petals, and other organs of aromatic herbs.[6] The pectinised cuticle and the cell walls of the glands, the constituents of which exhibit very low solubility in compressed CO_2, are the main intraparticle resistances controlling the extraction of essential oils. To disrupt these glands and liberate the entrapped oils, a pretreatment of the matrix is often performed prior to CO_2 extraction. Pretreatments applied to herbaceous matrixes are normally restricted to particle size reduction by mechanical means. However, during mechanical pretreatments, losses by degradation (oxidation and thermal degradation) and by evaporation of volatiles are observed, leading to a discrepancy between the essential oil composition of the herb and that of the extract. Comminution under cryogenic conditions is a promising technique because it minimizes both losses by evaporation and by degradation.[7] Other advantages claimed include a finer particle size and a total lower cost if the increased flavor strength of the extract is taken into account.[8]

In previous work, the physical disruption of essential oil glands by fast decompression of the CO_2 atmosphere involving the herbaceous bed was investigated.[9] The fast decompression treatment (FD treatment) involves a selective disruption of the essential-oil-containing glands. The efficiency of the FD treatment, studied at 310 K, was found to be strongly dependent on pre- and post-expansion pressures, decompression rate, and time of exposure to the preexpansion pressure. Exposure of a bed of intact material to 70 bar for 60 min followed by decompression to atmospheric pressure at a rate of 2 kg m^{-3} s^{-1} (expressed as the rate of CO_2 density drop in the bed) resulted in a very high efficiency of the FD treatment; 85% of the glands were estimated to be disrupted under these conditions.

The FD treatment, under the conditions mentioned above, was compared with mechanical treatments of the matrix. Comminutions under atmospheric and cryogenic conditions were used. The comminution of the material inside the extractor and under a closed atmosphere was also applied. The latter has the advantage of eliminating losses of essential oils by evaporation.

The different pretreatments were applied to oregano bracts prior to extraction with CO_2 under standard conditions. The rates and yields of extraction of essential oils and cuticular waxes were then compared with those obtained from an untreated matrix, and the losses of essential oils during the pretreatments of the matrixes were estimated.

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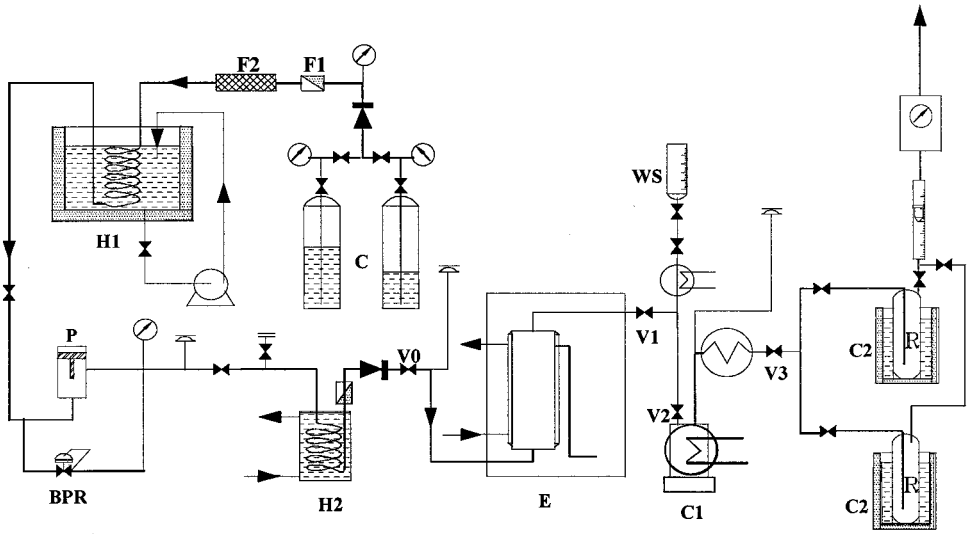

Figure 1. CO_2 extraction apparatus.

Materials and Methods

Matrix. Flowering heads from *Origanum virens L.* herbs were collected in Serra da Arrábida (Central West Portugal) after the flowering period and were sun-dried. The bracts were removed from the stalks and stored at low temperature (0−2 °C) in sealed polypropylene bags. A 500-g batch of bract material was prepared and used throughout the experimental tests. The tests were performed on this single batch within a short period of time (5 days) in order to minimize the discrepancies that can arise from nonuniformity or aging of the sample.

Matrix Pretreatments. *Atmospheric Comminution.* A commercial blender (Kenwood BL 350 dry grinder mill) was use to grind the oregano bracts. After comminution, performed under atmospheric conditions and with an exposure of 40 s to the blades, the matrix was exposed to the atmosphere for a 10-min period prior to its being transferred to the extractor (30 g of bracts was extracted). This procedure simulates a careless pretreatment of the matrix with little precaution to minimize essential oil losses.

Cryogenic Comminution. Oregano bracts were immersed in liquid nitrogen for 10 min. The frozen bracts were then transferred to the commercial blender and ground for 40 s. Immediately afterward, a 30-g sample was extracted with CO_2.

Internal Comminution. The internal comminution apparatus was designed to minimize losses observed in open comminution systems. A three-blade shaft was designed to fit the extractor vessel. The shaft, driven by an external electrical motor, was sealed with a PTFE O-ring on the base of the extractor. The intact bracts (15 g) were placed inside the extractor containing the customized blender. The comminution of the bracts, performed within the closed extractor, was immediately followed by CO_2 extraction. The design limitations of the customized blender resulted in a less efficient reduction of the particle size than that obtained from the commercial blender. On this basis, the comminution period was increased to 300 s (instead of 40 s). Despite this increase, the particle size obtained was still about 3 times greater than that obtained in the commercial blender (970 μm vs 360 μm).

FD Treatment. Fifteen grams of intact bracts were placed in the extractor. The bed was pressurized with CO_2 to 70 bar (at 310 K) and exposed to such conditions for 60 min before decompression to atmospheric pressure at a rate of 2 kg m^{-3} s^{-1}. The sample was then allowed to remain at atmospheric pressure for 10 min before the extraction with CO_2 was started.

Matrix Characterization. Samples of the bracts before and after each pretreatment were prepared and characterized in terms of particle size distribution, apparent density, and water content. The particle size was obtained through sieving of the samples. They were sieved using British Standard BSS 410 wire-mesh sieves coupled to a Retsh VS 1000 sieve machine. The apparent density was obtained by weighing the normally packed particles in a given volume, and the water content was measured in a "Dean and Stark" apparatus.

SEM micrographs were obtained from particles before and after the pretreatment and also after the extraction with compressed CO_2. These micrographs were used to observe physical changes in structure of the matrixes during the matrix pretreatments and CO_2 extraction.

Extraction Tests. The extraction tests with compressed carbon dioxide were performed at 100 bar, 310 K, and 0.25 kg/h. The apparatus used for CO_2 extraction is shown in Figure 1. A bed of material was enclosed in the extractor vessel (E). The diameter and height of the bed were 45 and 95 mm, respectively. Carbon dioxide [99.995% purity, BOC grade N4.5(CP)] was supplied from the storage cylinders (C) and was cooled in H1 before pressurization by a liquid pump (P). The extraction pressure and temperature, 100 bar and 310 K, were controlled by the back-pressure regulator (BPR) and the heater H2, respectively. The solvent was fed at the bottom of E and passed through the bed of material. Vessel E was maintained at the extraction temperature by a water jacket and an air bath. The stream containing the dissolved material left E and was expanded to 8−10 bar at V2 before entering the first collector (C1) where most solutes deposited into an extract holder contained in it. C1 was maintained at near ambient temperature by a heating cord. The stream leaving C1 was heated to 40−45 °C before being expanded to atmospheric pressure at valve V3 and entering one of two cold collectors in parallel (C2). These collectors were immersed in a dry ice/acetone mixture (−85 °C) to entrap water and any volatiles that may have escaped

Table 1. Matrix Characteristics before and after the Pretreatments

pretreatment	particle size (μm)	apparent density (kg m^{-3})	water content (% w/w)
FD treatment	1493[a]	62[a]	8.6[b]
internal comminution	970	114	8.6[b]
cryogenic comminution	250	293	7.8
normal comminution	360	227	6.6
no treatment	1507	63	8.6

[a] Result identical to that for the untreated matrix. Deviation is within the experimental error. [b] Water content was assumed to be that of the untreated sample as the pretreatment was performed inside the closed extractor.

from C1. V3 was adjusted to the desired flowrate, 0.25 kg/h, which was monitored by a rotameter and a gas flowmeter before the discharge of the CO_2.

The extract was collected at intervals as extraction proceeded. For each collection, valve V1 was closed, and the downstream pipe to C1 was quickly washed with hot acetone from buret WS. The dissolved extract from C1 was mixed with that from C2 in a given volume of acetone and retained so that the essential oils and cuticular waxes contained in it could subsequently be analyzed. The extract holder from C1 was replaced, and the use of the other cold collector C2 allowed for the dynamic extraction to proceed. Careful design of the collecting system and procedure resulted in an efficient and rapid collection of the extract (2−3 min) minimizing disruption to the extraction process.

After 200 min of dynamic extraction, the extractor was slowly depressurized, and the residual matrix from the CO_2 extraction was immediately hydrodistilled in order to estimate the residual content of essential oils. The content of essential oils in the matrix, after the matrix pretreatment and prior to CO_2 extraction, was then obtained by adding the final CO_2 yield to the hydrodistillation yield. Consequently, it was possible to estimate the losses of essential oils during the different pretreatments.

Analysis of the Extract. Immediately after its collection, the extract was dissolved in a 100-mL acetone solution. A 20-mL sample was taken and used for quantitative analysis of the essential oils by gas chromatography (GC). The remaining 80 mL of solution was allowed to evaporate in an air-circulated oven (45−50 °C) for evaluation of the mass of cuticular waxes. During evaporation of the acetone, part of the essential oils was also lost, and the quantification of the residual essential oils in the dried extract became important for the evaluation of the masses of cuticular waxes. Consequently, the dried extract was weighed and redissolved in acetone for quantitative GC analysis of residual essential oils. The mass of cuticular waxes, nonvolatile material co-extracted with essential oils, was then calculated.

The analysis of the essential oils was performed on a DB-5 capillary column (length, 30 m; i.d., 0.32 mm; film thickness, 0.25 μm). An Ai Cambridge gas chromatograph model GC 94, equipped with a flame ionization detector (FID), was used.

Results and Discussion

Matrix Characterization. Table 1 presents the characteristics of the untreated and pretreated matrixes. The particle size and apparent density of the matrix after the FD treatment remained, within the experimental error, unchanged. A very inefficient reduction of the particle size was obtained by the internal comminution apparatus; the particle size was even closer to that of the untreated matrix than to those obtained by the other comminutions performed in the commercial blender. The smallest particle size (and highest apparent density), observed from cryogenic comminution, was expectable from the brittle state of the matrix at such conditions. Losses of moisture were more significant during normal comminution. In the FD-treated and internally comminuted matrixes, the water content was assumed to remain unchanged as the pretreatments were performed in a closed atmosphere.

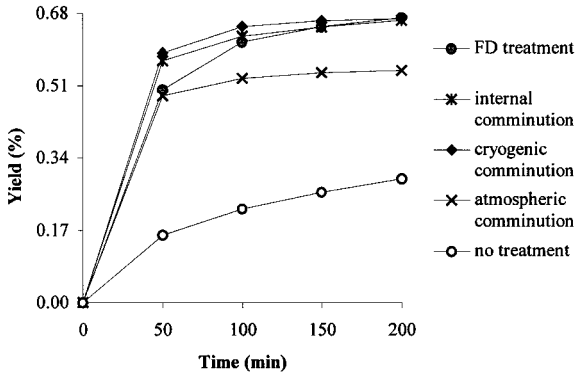

Figure 2. Essential oil extraction curves from differently treated matrixes.

Extraction of Essential Oils. To test the effect of the matrix pretreatment on the CO_2 extraction of essential oils, the extraction curves from differently treated matrixes were compared to that of the untreated matrix. Figure 2 shows the essential oil extraction curves. The yields are expressed as the percentage ratio between the mass of essential oils extracted and the initial mass of the water-free sample. It can be seen that there was a significant improvement in the extraction yields for extraction from pretreated matrixes. The very limited extraction rate observed from the untreated matrix resulted from strong intraparticle resistances dictated by the intraglandular location of the essential oils. The ability of CO_2 to penetrate intact glands is restricted by the low solubility of both cuticle and cell wall components. (Figure 3 presents a gland from the untreated matrix after 200 min of CO_2 extraction. In this case, the solvent was able to access the entrapped essential oils through a small pore, which was probably formed by the dissolution of part of the cuticle.)

From Figure 2, two different shapes of extraction curves can be distinguished among the treated matrixes. Mechanically treated (comminuted) matrixes revealed high extraction rates in the earliest 50-min period that were followed by a steep decrease, leading to very low extraction rates in the latter stages. On the other hand, the physically treated (FD-treated) matrix presents a smoother curve throughout the extraction. The difference between the shapes of the curves most probably arose from the extent of the damage inflicted to the glands and from the degree of displacement of the essential oils during mechanical and physical pretreatments. The disruption of glands during the comminution of the bracts occurs either by direct contact with the blades of the blender or by particle collisions (interparticle and particle/blender surface collisions) promoted by the turbulence within the blender. The location of the glands on the surface of the bracts makes them very

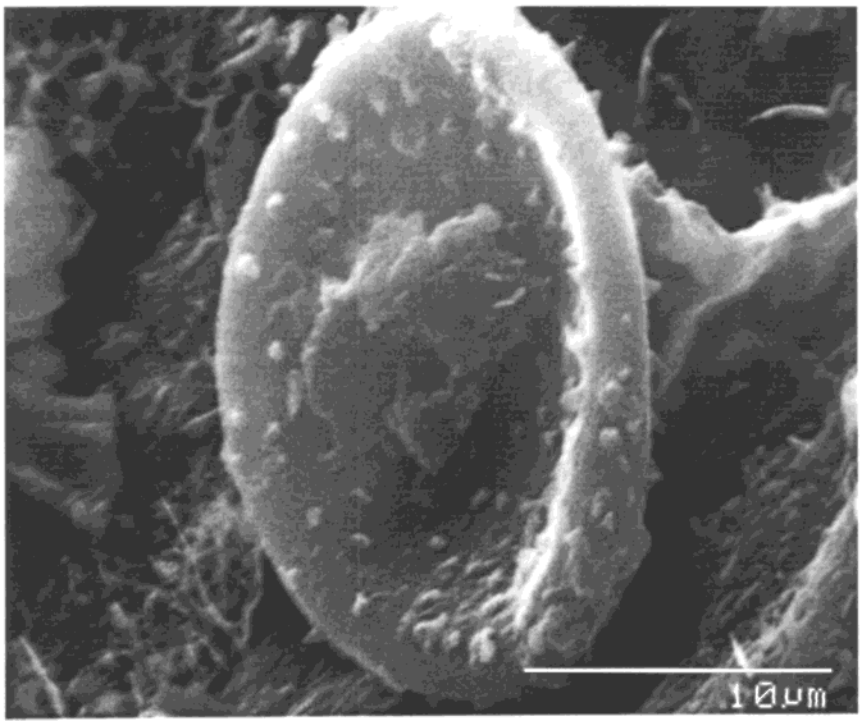

Figure 3. Scanning electron micrograph of a gland from the untreated matrix after 200 min of CO_2 extraction (×4000) (a pore in the cuticle allowed access to the essential oils).

Table 2. Estimation of Essential Oils Losses during the Pretreatment of the Matrix

	yields (% of initial dry mass of sample)			
pretreatment	CO_2 extraction	hydrodistillation	initial content	losses[a] (%)
FD treatment	0.67	0.10	0.77	–
internal comminution	0.66	0.06	0.72	6
cryogenic comminution	0.67	0.06	0.73	5
normal comminution	0.55	0.05	0.59	23

[a] As a percentage of the initial content in the FD-treated matrix.

vulnerable to such collisions. Experimental evidence of gland disruption by particle collisions includes the difference in magnitude between the particle size of comminuted bracts (250−970 μm) and the size of the glands (5−20 μm) and the surprisingly good results obtained from the internally comminuted matrix. In the latter case, the inefficient particle size reduction seems to have been greatly compensated for by an increase in the comminution period and thus in the number of particle collisions. During the FD treatment, by contrast, the disruption of the glands resulted from a pressure gradient formed across the gland during the fast decompression of the bed. The pressure gradient arose from the limited ability of the glands to discharge the CO_2 accumulated in their interior during the period of exposure to the preexpansion conditions.[9] Microscopic observations revealed a larger proportion of intact glands in mechanically treated matrixes. However, the high turbulence generated during comminutions is likely to have resulted in greater displacement of the essential oils from disrupted glands. This fraction of readily accessible oil was easily extracted in the earliest stage, whereas the remaining intraglandular oil was very slowly extracted in the later stages. The steep decrease of the extraction rate observed in mechanically treated matrixes is, therefore, probably related to the exhaustion of the readily accessible oil fraction. The absence of significant turbulence during the FD treatment resulted in lower displacement of the essential oils and, consequently, in a more gradual extraction curve.

After the 200 min of extraction allowed, very similar yields were obtained from FD treatment, internal comminution, and cryogenic comminution, specifically, 0.67%, 0.66%, and 0.67%, respectively. The final yield obtained from atmospheric comminution, 0.55%, was compromised by the extent of losses of essential oils during the pretreatment. (The ultimate yields reflect not only the efficiency of each treatment in liberating and displacing the intraglandular essential oil but also the losses of essential oils during the pretreatments.)

The losses of essential oils during the pretreatments were evaluated by comparison of the essential oil contents in the treated matrixes prior to CO_2 extraction. To estimate the initial content in the matrixes, the final CO_2 extraction yields were added to the yields obtained by hydrodistillation of the residual matrixes. The losses of essential oils were then calculated in relation to the initial content in the FD-treated sample. (Losses by evaporation, thermal degradation, and oxidation are virtually eliminated in the FD treatment by use of a closed, homogeneous, low-temperature, and inert CO_2 atmosphere.) Table 2 presents a summary of these results.

It is seen from Table 2 that all comminutions revealed essential oil losses when compared to the FD pretreatment. In the case of normal comminution, where no attempt was made to minimize the losses, they accounted for 23% of the initial content. These losses were reduced to 6% and 5%, respectively, when internal and cryogenic comminutions were applied.

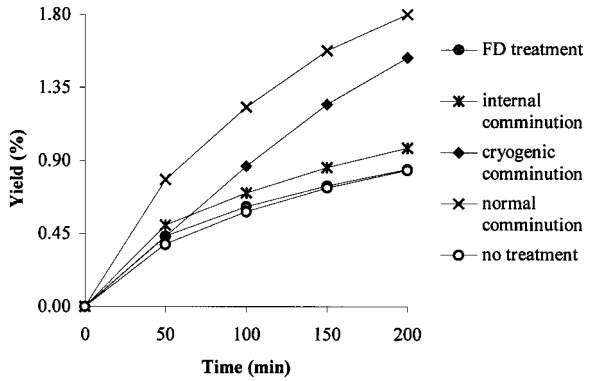

Figure 4. Cuticular waxes extraction curves from differently treated matrixes.

(It should be noted that the yield obtained by hydrodistillation of an untreated matrix accounted for 0.75%, in close agreement to the estimated initial content within the FD-treated matrix, i.e., 0.77%. The latter was obtained by summing the yields from CO_2 extraction and hydrodistillation of the residual FD-treated matrix, as seen in Table 2.)

Extraction of Cuticular Waxes. The cuticular wax extraction curves are shown in Figure 4. The mass percentage yields are, as before, expressed in terms of dry mass of sample used. The lowest extraction rates were obtained from the untreated and FD-treated matrixes. Slightly higher extraction rates resulted from the internally comminuted matrix, whereas those obtained from both cryogenically and atmospherically comminuted matrixes were significantly higher. In a recent study[10] in which the effect of the CO_2 flowrate was investigated, the extraction of cuticular waxes from similarly pretreated matrixes was found to be mostly dependent on the mass of CO_2 used and, consequently, related to the solubility of the waxes in the compressed solvent. The differences observed in Figure 4 should, therefore, be addressed to the availability of cuticular waxes solutes after the pretreatments and to the selectivity of CO_2 toward more soluble components. A higher availability of solutes results in an extended selective extraction of more soluble components and thus in higher extraction rates. The selective extraction of cuticular wax components arises from the complex composition of the available cuticular waxes solutes. In intact herbaceous matrixes, the available solutes are largely composed of the hydrophobic wax layer (epicuticular waxes) that covers the surfaces of plants. (Hydrocarbons, β-diketones, fatty acids esters, fatty alcohols esters, free fatty acids, and free fatty alcohols were found among the main components of epicuticular waxes.[11]) In addition, solutes present in internal tissues may become available during the pretreatment of the matrix.

In general, the smaller the particle size obtained from the pretreatment, the greater the amount of internal solutes made available. The relatively low extraction rates observed from the internally comminuted matrix are thus to be expected in view of the inefficient reduction in particle size during this matrix pretreatment. However, in a comparison of the extraction curves from cryogenic and atmospheric comminution, the particle size relation is contradicted. Despite the smaller particle size of the cryogenically comminuted matrix, a lower extraction rate was observed. A plausible explanation is a lower displacement of cuticular wax solutes during low-temperature pretreatments; under cryogenic conditions, the solid state of the solutes restricts their displacement over the surfaces of the matrix, limiting the rates of extraction obtained.

In contrast with comminuted matrixes, the FD-treated matrix did not reveal any enhancement in the rate of extraction observed from the untreated matrix; the extraction rates were coincident. This physical treatment seemed only to affect the essential oil glands. Observations of the untreated and FD-treated matrixes, also suggest the specificity of the FD treatment. Visually, the FD-treated bracts were indistinguishable from the untreated bracts, and even under a microscope, only the minute glands appeared disrupted on the FD-treated bracts (see Figure 5)

Extract Composition. Figure 6 presents the evolution of the cumulative composition of the CO_2 extract. The extract composition is presented as percentages of

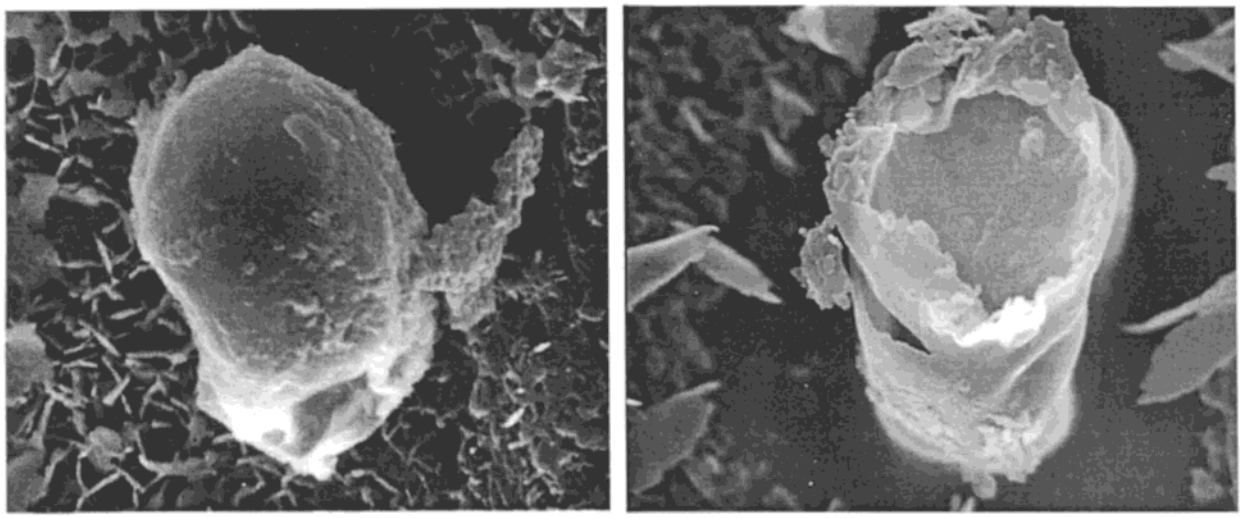

Figure 5. Scanning electron micrographs: (a) intact gland from untreated sample (×4000), (b) disrupted gland after FD treatment (×6000).

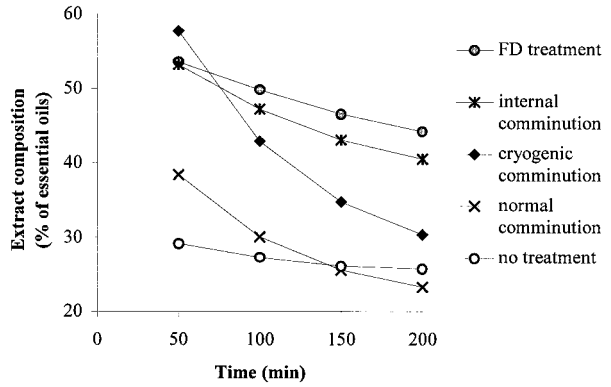

Figure 6. Cumulative extract composition during CO_2 extraction.

essential oils in the water-free extract. It can be seen that the essential oil content in the extract decreased as extraction proceeded. In addition, this decrease was more pronounced in the matrixes with smaller particle sizes (the cryogenically and atmospherically comminuted matrixes). The more concentrated extracts were obtained from the FD-treated and internally comminuted matrixes (44% and 40% after 200 min, respectively). The atmospherically and cryogenically comminuted matrixes presented final essential oil contents of only 23% and 30%, respectively, whereas in the intact matrix, the essential oils accounted for 26% of the water-free extract.

Conclusions

An efficient pretreatment of the herbaceous matrixes is required if acceptable yields and rates of extraction of essential oils are to be obtained using compressed CO_2 as a solvent. Moreover, the matrix pretreatment was found to have a significant effect on the extent of co-extraction of undesirable cuticular waxes components and, therefore, on the extract composition.

The efficiency of a matrix pretreatment as a prelude to CO_2 extraction of essential oils depends not only on its ability to liberate and displace the intraglandular essential oils, but also on the extent of essential oil losses during the process. The efficiency of each pretreatment can be assessed by comparison of the essential oil yields from a subsequent CO_2 extraction and from the estimation of the losses during the pretreatment. Cryogenic comminution, internal comminution, and FD treatment were all found to be efficient matrix pretreatments for CO_2 extraction of essential oils; after 200 min of CO_2 extraction, the yields obtained were 0.67%, 0.66%, and 0.67%, respectively. These are substantially higher than the 0.55% and 0.29% obtained from atmospherically comminuted and untreated matrixes, respectively. The comparatively low yield obtained from atmospheric comminution of the matrix resulted from the significant losses observed in this careless pretreatment. The losses of essential oils were estimated in relation to the initial content in the FD-treated matrix. (Losses of essential oils during the FD treatment were virtually eliminated by a closed, homogeneous, low-temperature, and inert CO_2 atmosphere.) Twenty-three percent of essential oils was lost from a matrix exposed for 10 min to atmosphere after atmospheric comminution. These losses were significantly reduced by avoiding the exposure to atmosphere after cryogenic and internal comminution of the matrixes (only 5% and 6%, respectively, were lost). These results suggest that essential oils of a better quality are obtained after FD treatment of the matrixes. Another advantage of this method is that it selectively liberates the essential oils in relation to cuticular waxes. This results in a final extract with lower wax content. On the other hand, the FD treatment is time-consuming and limits the amount of material that can be loaded into the extractor.

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