Extraction of essential oils from Algerian myrtle leaves using instant controlled pressure drop technology

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ABSTRACT

In the present work, the new extraction process of Détente Instantanée Contrôlée DIC (French, for instant controlled pressure drop) was studied, developed, quantitatively and qualitatively compared to the conventional hydrodistillation method for the extraction of essential oils from Algerian myrtle leaves. DIC was used as a thermomechanical treatment, DIC subjecting the product to a high-pressure saturated steam. The DIC cycle ends with an abrupt pressure drop towards vacuum, and this instantly leads to an auto-vaporization of myrtle volatile compounds. An immediate condensation in the vacuum tank produced a micro-emulsion of water and essential oils. Thus, an ultra-rapid cooling of residual leaves occurred, precluding any thermal degradation. An experimental protocol was designed with 3 independent variables: saturated steam pressure between 0.1 and 0.6 MPa, resulting in a temperature between 100 and 160 °C, a total thermal processing time between 19 and 221 s, and between 2 and 6 DIC cycles. The essential oils yield was defined as the main dependent variable. This direct extraction gave high yields and high quality essential oil, as revealed by composition and antioxidant activity (results not shown). After this treatment, the myrtle leaves were recovered and hydrodistilled in order to quantify the essential oil content in residual DIC-treated samples. Scanning electron microscope (SEM) showed some modification of the structure with a slight destruction of cell walls after DIC treatment.

1. Introduction

The common myrtle, *Myrtus communis* L., belongs to the Myrtaceae family which includes more than 5650 species that are known to be rich in essential oils [1]. It is a small wild shrub, common in the Mediterranean regions. Its evergreen and aromatic leaves are quite resistant to hot and cold weather.

Myrtle is often found in oak, mastic and Aleppo pine forests, which require limestone and/or silica soils. In Algeria, it is present in Tell on the slopes of hills and on coastal areas from east to west, sometimes in remote areas. The desert species, *myrtus nivellei*, is commonly found in Hoggar and Tassili. Its leaves are highly appreciated by the Tuareg as herbal medicine [2].

Like many natural products, after a period of decline there has been a renewed interest in the myrtle. Its bluish black berries are edible when fresh. They are also used to make alcoholic beverages [3] or as a substitute for hops in beer production [4].

Myrtle plants emit a pleasant perfume when the leaves and flowers are crushed. This perfume is mainly due to essential oil molecules which are stored in the secretory cells located in the leaves, flowers and berries [5–8]. Its leaves and flowers are highly prized in perfumery [5] and in some cosmetic formulations.

Myrtle is now one of the main medicinal plants in Algeria. Many authors have reported that the myrtle and its essential oils have a great potential as a medicinal plant, having hypoglycemic [9], antimicrobial [10], antiseptic and anti-inflammatory [11,12], antimutagenic [13] and antioxidant [14] properties. Its leaves are used to treat stomach ulcers, urinary tract infections and dysentery. Myrtle is mainly used as an expectorant and in pulmonary inhalation of the vapors produced after leaf decoction.

The large variability in the chemical composition of its essential oils has attracted many researchers and has been the subject of many studies [6,15–23].

The present study of the Algerian myrtle was interesting to undertake for two main reasons; on the one hand,

it is widely available and frequently used as a medicinal and aromatic plant in Algeria; on the other hand, there has been no mention of the volatile compounds of the essential oils of the Algerian myrtle in the literature, as far as we know.

The industrial scale extraction of essential oils is largely artisanal and still uses varieties of steam extraction. Used since antiquity it has slightly progressed in terms of kinetics, energy consumption and pollution impacts. During hydrodistillation, water and acidity [24], as well as long heating can induce hydrolysis reactions [25] and also rearrangements, isomerization and racemization reactions, and oxidative changes to natural compounds [26]. Hydrodistillation, which uses both heating and cooling systems for the evaporation and condensation of volatile compounds involves high-energy consumption and has serious impacts on the environment. It uses about 4 kWh/kg of raw material, which corresponds to 3.360 kg CO_2 released into the atmosphere [27]. This energy costs, coupled with a lack of automation and a high degree of degradation of the final waterlogged residual solid, as well as a possible degradation of essential oil molecules, renders hydrodistillation unattractive from an economic point of view.

Processes such as organic solvent extraction, with or without ultrasound treatment, have been used. However, it is difficult to completely remove the traces of solvent from end-extracted products and the highest level admitted by the international standards is increasingly restrictive [28]. The final distillation stage would also lead to a great loss of light compounds.

Other extraction processes such as microwave (MW) assisted extraction have been proposed [29,30]. They offer some advantages regarding kinetics and preservation of the chemical composition [31], but as far as we know, no industrial development has been achieved and all applications are still at the laboratory or pilot scale. Other operations as the supercritical have been used [32]. Although attractive with low operating costs, the disadvantages of this extraction process are the costly facilities required and a cumbersome installation. Thus, it is only adopted for high added-value products [33]. Note that the use of supercritical CO_2 SCF as a solvent is not selective and can extract not only volatile molecules but also other unwanted compounds such as waxes and resins that must then be eliminated [34].

In this context, it was proposed to carry out a comparative study of the extraction of the essential oils of the Algerian myrtle using two different methods: traditional hydrodistillation and the innovative technology of Détente Instantanée Contrôlée DIC (French, for instant controlled pressure drop). DIC was defined in 1988 [35] and has been studied, developed, optimized and used at industrial scale for various applications like drying and decontamination [36], texturing [37], the extraction of non-volatile molecules such as flavonoids [38] and of volatile compounds of some plants such as cananga flowers [39].

Regarding volatile compounds, the DIC extraction method has been very relevant, in terms of process performance and attributes of the final product. The most instructive study was reported in the work of Kristiawan et al. [39] who extracted the essential oils of Indonesian Kananga in less than 6 min with a yield of 2.8 g/100 g dry matter compared with a similar yield (2.5 g/100 g dry matter) achieved with steam distillation but which took 16 h. These previous studies encouraged us to try to assess the feasibility of using DIC as a method to extract the essential oils from the myrtle. We opted for a comparative study of the extracts obtained by direct DIC extraction (DIC-EO), and by hydrodistillation (HD-EO); the latter was carried out on raw material and on residual DIC-treated solids.

2. Materials and methods

2.1. Reagents and chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH•) was obtained from Sigma–Aldrich (Saint-Quentin Fallavier; France) and analytical grade methanol was obtained from Carlo Erba (Val de Reuil; France.).

2.2. Plant material

Raw material was harvested during the period of fruit development of the myrtle. The aerial parts (leaves, berries, branches and stems) were picked very early, in mid-November 2007 in the forest of Bainem, located in the northwest suburbs of Algiers at 400 m altitude. The plant was identified by the herbarium of the National Institute of Agronomy (INA) of Algiers.

The first pretreatment involved separating the leaves from the branches, stems and berries. In this study, only the leaves were used; they were subjected to partial and gradual drying, followed by manual cutting. Moisture content was measured using the desiccation method in a Mettler Toledo LP-16 Infrared Dryer/Moisture Analyzer with a Mettler Toledo PE360 Balance (Bishop International Akron, OH, USA). These measurements of water content, repeated with four samples, were conducted on a 3 g of myrtle leaves, coarsely chopped and placed in a thin layer glass capsule and dried in the oven at 105 °C for 24 h until a constant weight was reached. The initial moisture content was determined as $48.4\% \pm 0.2\%$ wb (wet basis), which is 93.8 ± 0.75 g $H_2O/100$ g dry matter.

2.3. Protocol of treatment

In the present study, the treatment design was achieved as illustrated in Fig. 1.

2.3.1. Drying process

Fresh myrtle leaves were let to dry for 48 h after harvesting using a 29 ± 1 °C/1 ± 0.2 m s^{-1} air drying system. Low temperature drying was adopted in order to preserve the volatile compounds as much as possible. The aim was to reach a final moisture content of 16.2 ± 0.5 g $H_2O/100$ g dry matter, which is generally adequate for DIC treatment as indicated by some preliminary work. In the cases of myrtle leaves, DIC treatment of rehydrated material that had been completely dried did not give convincing results; the moisture content needed for DIC treatment must be reached only through a desorption process. Indeed, the specific structure of the leaves with a thick, waxy cuticle may confer some resistance to water absorption after drying (waterproof surface).

2.3.2. Instant controlled pressure drop DIC equipment

DIC reactor has been presented in numerous papers [40]. The reactor we used was a 7 l processing vessel with a heating jacket; thermal treatment in this vessel is achieved using saturated steam with a pressure varying from 5 kPa up to 1 MPa, which means a temperature between 33 and 159 °C. A 0.7 m^3 vacuum tank with cooling water jacket is connected with a water ring vacuum pump allowing the vacuum to be maintained at 5 kPa. A pneumatic valve ensures an "instant" connection between the vacuum tank and the processing vessel; it can open in less than 0.2 s. Some other valves control the flow of steam and compressed air within the processing vessel.

2.3.3. DIC treatment

DIC cycle is usually defined as a rapid (usually between 5 and 60 s) thermal treatment with high-pressure saturated steam at
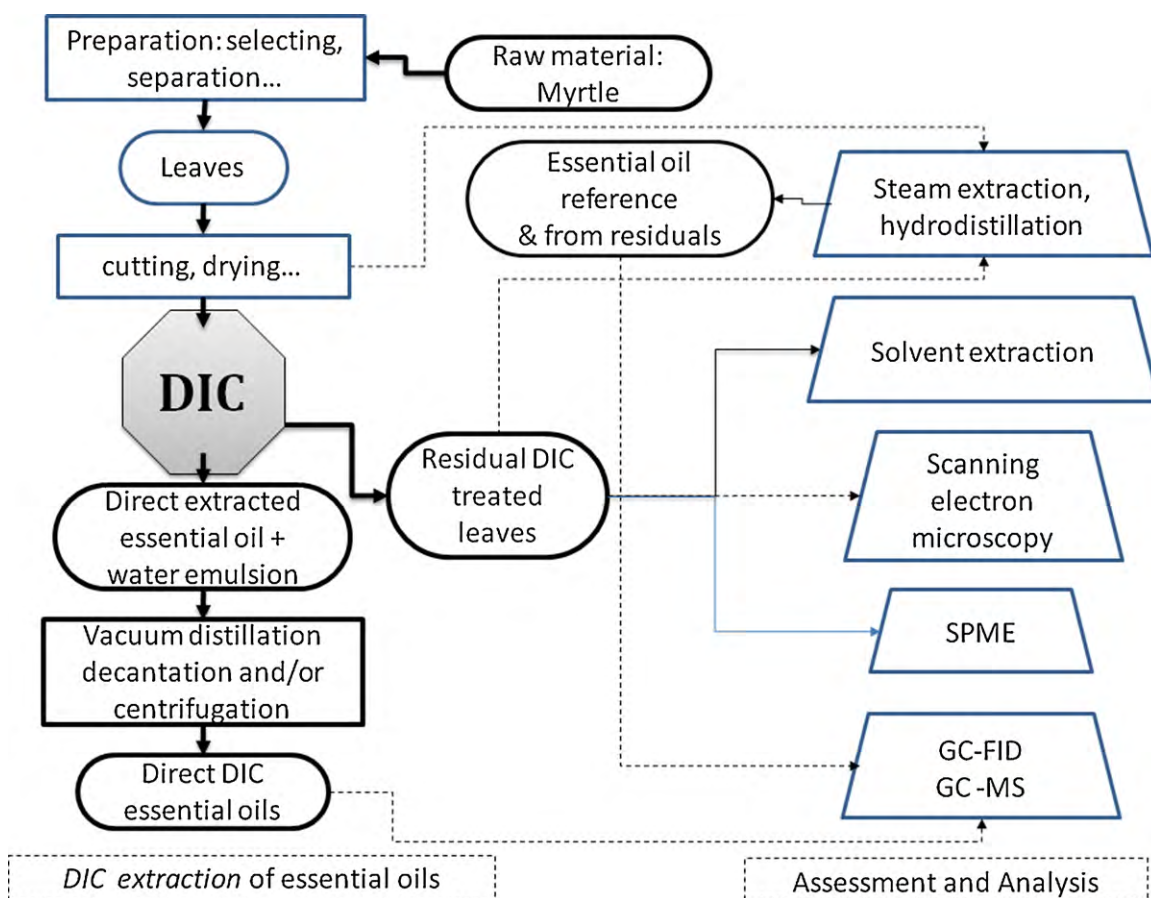
Fig. 1. Protocol for the extraction of essential oils (EO) from Algerian myrtle leaves using instant controlled pressure drop (DIC) treatment as compared with hydrodistillation.

about 0.1–0.7 MPa depending on the product and the needed operation. This high temperature–short time stage is followed by an instant pressure drop towards a vacuum at about 5 kPa inducing a mechanical effect. Such an abrupt pressure drop, at a rate $\Delta P/\Delta t$ higher than $0.5\,\text{MPa s}^{-1}$, simultaneously provokes an autovaporization of a part of the water in the product, and an instantaneous cooling of the products, which stops thermal degradation. Depending on the severity of the treatment, the structure can be slightly or greatly modified. Also, the operating conditions can preserve or break, the cell walls.

The DIC extraction used here with Algerian myrtle leaves involved subjecting the material to various DIC cycles. Extraction was achieved through evaporation and autovaporization phenomena resulting from thermal and thermomechanical effects. The ranges in number of DIC cycles, saturated steam pressure, and total treatment time (Table 1) were defined after some preliminary experiments. The sample was placed in the treatment vessel and a vacuum (about 5 kPa) established to remove the air present in the vessel, allowing close contact between the surface of the sample and the steam that was injected just after. In this study, after the last DIC cycle, atmospheric pressure was restored in the treatment vessel to take the sample off [39]. Fig. 2 presents a flow diagram of this protocol. Thermal treatment (phase d), may involve a partial evaporation of the essential oils; autovaporization of a part of the water and the volatile molecules (essential oils) has to be strictly linked to the instant pressure drop towards a vacuum, which would involve a mechanical expansion, as well as instant cooling of the residual solid which would prevent thermal degradation [39].

The present study included two main stages. Preliminary experiments were performed to define the range of various operating parameters such as steam pressure, total thermal treatment time and number of cycles.

In the second stage, DIC extraction was carried out using a specific experimental design to identify and compare the effects of total thermal treatment time and the number of cycles, chosen as operating parameters (or independent variables). This was done to ascertain the relative impacts of the evaporation and autovaporization phenomena, respectively. The extracted product was collected as an emulsion of water and essential oils from the vacuum tank. The amount of essential oils present in this emulsion was quantified and defined as the yield of the "direct DIC extraction" and was one of the main dependent variables (responses) of the operation.

2.3.4. Hydrodistillation

The pilot plan hydrodistillation equipment used in the present study was a modified Clevenger. Some modifications were made to the equipment in order to carry out various treatments and quantify the amount of essential oils extracted. 200 g of coarsely chopped leaves were immersed in 2 l of distilled water in a 3 l distillation flask. The extraction of essential oils was carried out over 3 h, from the first drop of distillate until the plant material had

Table 1
Independent variables used in RSM (response surface methodology) for DIC treatment of Algerian myrtle leaves with an initial water content maintained at 16.2 g $H_2O/100$ g dry matter obtained by desorption.

Coded level	$-\alpha$	-1	0	1	$+\alpha$
$X_1 = P$ Saturated steam pressure (MPa)	0.1	0.2	0.35	0.5	0.6
$X_2 = t$ Total thermal treatment time (s)	19	60	120	180	221

α (axial distance) $= (2^k)^{1/4}$, where k is the number of independent variables. In the present case: $k = 3$ and $\alpha = 1.681793$.
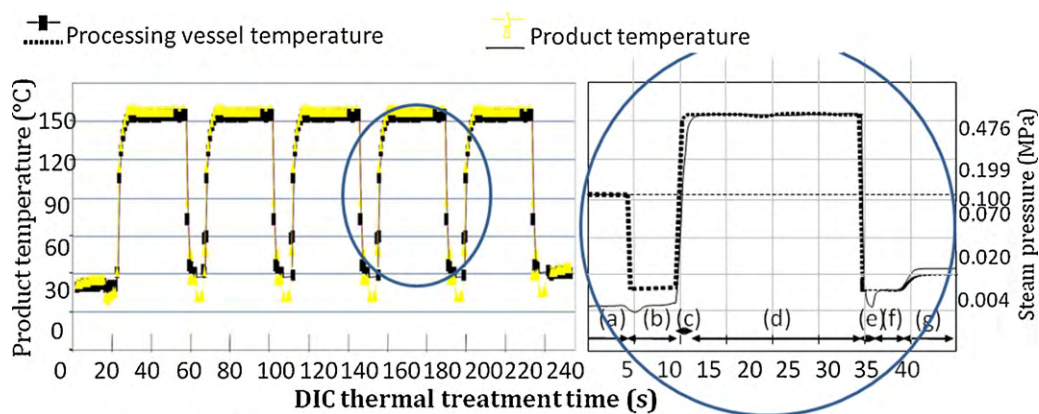
Fig. 2. Temperature and pressure history of a DIC extraction cycle: P_A is the steam pressure in the autoclave, P_V the pressure in the vacuum tank, T_A the temperature in the autoclave, T_P the temperature of the product. (a) Atmospheric pressure; (b) initial vacuum; (c) saturated steam injection to reach the selected pressure; (d) constant temperature corresponding to saturated steam pressure; (e) abrupt pressure drop towards a vacuum; (f) vacuum; (g) release to atmospheric pressure.
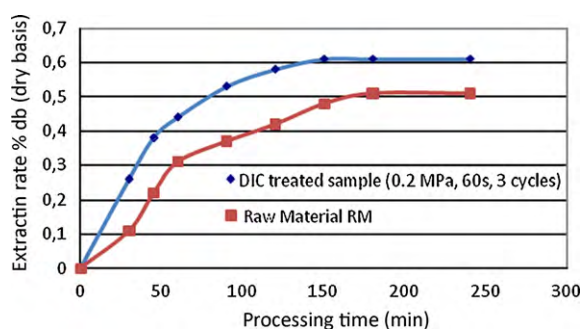

Fig. 3. Kinetics of hydrodistillation of Algerian myrtle leaves either untreated (raw material) or treated by instant controlled pressure drop (at 0.2 MPa steam pressure, for 60 s and with 3 cycles).

been completely consumed. The duration of extraction was determined before by studying the kinetics of the operation using the measurement of the quantity of essential oils versus time (Fig. 3). The oil recovered and dried with anhydrous sodium sulfate was then stored in a refrigerator at 4 °C in a tightly closed amber vial, away from sources of degradation (heat or light, etc.), for further analyses.

Hydrodistillation was carried out on the raw material as well as on DIC-treated residual solids. The aims of these operations were (1) to compare the relative yields of essential oils directly extracted after different DIC treatments with the hydrodistillation yield as a reference, and (2) to estimate the amount of essential oils inside the DIC-treated leaves. The operation was performed three times, to test repeatability.

2.4. Statistical and experimental design protocol

The RSM (response surface methodology) was developed by several authors [41]. In the present work, this experimental design was defined after some preliminary trials to test the behavior of the plant (structure expansion, thermal degradation, etc.) versus the operating parameters. The results of these preliminary experiments and other previous work [39] allowed us to identify the effects of each factor and target the optimal extraction conditions with the lowest number of trials and the most relevant responses. We only selected three factors that seemed to have a significant influence in essential oil extraction. These were saturated steam pressure, number of cycles, and total thermal processing time. They ranged from 0.1 to 0.6 MPa, 2 to 6 cycles, and 19 to 221 s, respectively (Table 1). Other parameters, such as the final pressure at the vacuum level in

the vacuum tank and the volume ratio between the treatment vessel and the vacuum tank, were kept constant. The initial material moisture content just before DIC treatment was also kept constant (16.2 g H_2O/100 g dry matter obtained using a desorption process). The main objective was to identify the conditions yielding the highest amount of DIC extracts with the possibility of maximizing the information obtained from a small number of experiments. The 22 trials of this experimental design were run randomly to minimize the effects of an unexpected variability of responses due to unrelated and uncontrolled factors. The dependent variable concerned process performances (in terms of efficiency) as well as the composition of the final extracted (non shown results).

The statistical analysis of the results was carried out using the analysis design procedure of Statgraphics Plus software for Windows (1995, version 5.1, Levallois-Perret, France). Variance (ANOVA) was performed to determine significant differences between independent variables ($P \leq 0.05$). Pareto charts were introduced together with response surface, empirical model coefficients and R^2 were determined. The response parameter (dependent variable Y) was expressed through a second-order polynomial empirical model of independent variables:

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n} \beta_{ij} x_i x_j + \varepsilon$$

where β_o, β_i, β_{ii} and β_{ij} were the regression coefficients, x_i were the independent variables, ε was random error, i and j were the indices of the factors. Response surface methodology can be used to optimize the operating parameters by coupling various studied responses [39].

2.5. Assessment and response parameters

We considered yields of essential oils obtained directly from the vacuum chamber by DIC as a first response factor (dependent parameter). The amount of residual essential oils in the myrtle leaves after various DIC treatments was examined by conducting a 3 h hydrodistillation. Neither SPME analysis nor steam extraction was carried out in the present study.

2.5.1. Scanning electron microscope SEM

Micro-structures were observed using an environmental type JEOL 5410LV FEI Quanta 200F Scanning Electronic Microscope (SEM) (Philips Croissy-sur-Seine; France), at the Common Center of Analyses (CCA) of the University of La Rochelle. The samples were placed on a covered stud using carbon adhesive; the samples were

scanned in a partial vacuum (7 Pa) with an acceleration tension of 20 kV.

Changes in the myrtle leaf microstructure of raw material and DIC-treated samples were compared to highlight the impact of DIC as a texturing treatment.

2.5.2. Analysis by GC and GC–MS

The essential oils obtained by DIC (DIC-EO) and by hydrodistillation (HD-EO and HD-DIC-EO) were analyzed by gas chromatography with or without mass spectrometry.

2.5.2.1. Analysis by GC.
A Varian 3800 chromatograph (Cie Varian, Les Ulis; France) equipped with a Flame Ionization Detector (FID) and fused silica capillary columns, was used. Analyses of different stationary phases and identification of various compounds were performed with two brand Varian columns. One polar (polyethylene glycol) model CP-WAX 52CB column and various non-polar (polydimethylsiloxane) Factor Four VF-5 ms columns were used, with the same dimensions: 30 m long, 0.25 mm internal diameter and a 0.25 μm thick film of stationary phase.

The analytical conditions were as follows: the essential oils were diluted with hexane at about 1/10. The flow of the carrier gas helium was kept constant at 1 ml/min. The temperatures of injector and detector were 250 and 280 °C, respectively. The injected volume was 0.50 μl with a split of 1/20. The temperature was maintained at 50 °C for 8 min, then increased by a gradient of 2 °C/min to reach 250 °C; this temperature was kept constant for 5 min.

A series of n-alkanes from C5 to C28 was injected under the same analytical conditions as the samples, for the measurement of retention indices.

2.5.2.2. Analysis by GC–MS.
A Varian 3900 chromatograph (Cie Varian, Les Ulis; France) coupled to a Saturn 2100T mass spectrometer (Cie Varian, Les Ulis; France), with the same columns and the same analytical conditions were used and programmed.

Ionization and fragmentation were performed by electron impact at 70 eV, with an injection temperature of 250 °C. The temperatures of detection and transfer line were 280 and 250 °C, respectively.

The scanning range of m/z was from 35 to 400 uma, the data acquisition frequency was 1.2 scans/s. Identification of compounds was achieved by comparing a part of their retention index (Kovats indices determined from the retention time in the series of n-alkanes) with those of the reference; it was also carried out in part using their characteristic mass spectra ion-fragments and comparing them with those in the literature [42–46] or those listed in the databases and spectral libraries [Varian 1998 and Saturn NISTMS Database libraries]

Elution order and relative quantification (%) of the compounds were calculated from chromatograms obtained by GC-FID with the non-polar VF-5 ms capillary column.

3. Results and discussion

3.1. Comparative extraction yields of hydrodistillation and DIC

3.1.1. Hydrodistillation
The essential oil obtained from both DIC-treated and untreated (raw material) myrtle leaf samples, by hydrodistillation was very clear, with a strong and pleasant eucalyptol odor. It had a very light yellow color.

The average yield of essential oil extracted from the untreated myrtle leaves was 0.51 ± 0.04 g EO/100 g dry matter.

The global chemical analysis of the myrtle leaf essential oils is illustrated in Table 2. In this analysis, 79 compounds were identified representing 98.76% of the total chemical composition of myrtle leaf essential oils. Monoterpene hydrocarbons were the major components (55.54%) with α-pinene as the main compound (50.81%).

The oxygenated monoterpenes were present at 35.78% of which 24–32% represented by 1,8-cineole. Other compounds were present at lower levels, such as limonene (2.63%), α-terpineol, (2.51%), methyl-eugenol (2.33%), geranyl acetate (2.08%) and linalool (1.32%). However, the composition may depend on various factors and probably change with the harvesting season and the growth cycle of the plant [47], as well as the region [48] and the extraction method [49].

The chemical composition of the myrtle essential oils determined in this work differs from that reported from Tunisia, Lebanon, Corsica, Yugoslavia [48], Iran [50] and Italy [8]. Nevertheless, in all these oils, α-pinene was the major component.

Other investigations undertaken by Boelens and Jimenez [17], Jerkovic et al. [47] and Chalcat et al. [48] on myrtle from Spain, Morocco, Albania and Croatia found that myrtenyl acetate was the main compound. In other work it was mentioned that the major components were 1,8-cineole, linalool and myrtenyl acetate [51], limonene [52] and linalyl acetate [21].

3.1.2. Direct extraction of essential oils by DIC treatments
The DIC-EO essential oils obtained directly by DIC treatment of myrtle leaves exhaled a strongly spiced and particularly odoriferous fragrance. They had a light yellow color and were much more translucent than HD-EO (the hydro-distillated extract).

The highest total yield of 0.56 ± 0.12 g EO/100 g dry matter was obtained at 0.6 MPa saturated steam pressure, 120 s total thermal treatment time and with four cycles; this was slightly higher (+10%) than with hydrodistillation. The results of the volatile compounds analysis allowed us to identify 77 compounds, representing 98.32% of the total essential oil composition. Monoterpenes were found to be the major chemical compounds of the essential oils; oxygenated monoterpenes were the major component (39.75%), followed by the hydrocarbon monoterpene (26.46%). The major compound of the first category was 1,8-cineole (21.76%), whereas α-pinene was present at 23.33%. The main conclusion concerns the composition: the oxygenated compounds were richer and more varied for DIC-EO than HD-EO. Note that some compounds which were invisible or present at negligible levels in HD-EO were present at a significant level in DIC-EO. Thus the level of geraniol increased from 0.51% to 1.48%, α-terpinyl acetate from 0.85% to 1.22%, geranyl acetate from 2.08% to 3.83%, methyl-eugenol from 2.33% to 5.38%, and caryophyllene oxide from 0.63% to 4.21%. However, the most striking case was that of eugenol, which was not visible in HD-EO (<0.05%) but was present at 4.06% in DIC-EO. This increase can be explained by the hydro-solubility of eugenol. Indeed, in a study on essential oil extraction, Chemat et al. [53] indicated that eugenol, whose solubility in water is 2.52 g/l at 25 °C, can be found only at trace levels in the oil obtained by hydrodistillation. Furthermore, it was noted that Linalool increased from 1.32% to 2.94% and neryl acetate from trace levels to 1.23%. Other compounds lacking in HD-EO extracted essential oils were present at about 1% in DIC-EO. Thus methyl citronellate, carvacrol, trans-pinocarvyl acetate, and thymol were only present as traces in HD-EO but were present at 0.1%, 0.1%, 0.15%, and 0.2% in DIC-EO.

3.1.3. Hydrodistillation of residual essential oil in DIC-treated myrtle leaves
The hydrodistillation of myrtle leaves initially treated by DIC should not be considered as a process. This protocol was primarily designed to assess and identify the effectiveness of DIC by quantifying the amount of residual essential oils in the material after DIC treatment. The average amount of such residual essential oils varied between 0.005 ± 0.004 g of essential oils/100 g of dry matter and 0.61 ± 0.03%. The results showed a complete extraction of

Table 2
Main compound classes of essential oil extracted from Algerian myrtle leaves using hydrodistillation (HD-EO), instant controlled pressure drop (DIC-EO), and hydrodistillation of DIC-treated leaves (HD-DIC-EO).

Compound classes	HD-EO[a] (%)	DIC-EO[b] (%)	HD-DIC-EO[c] (%)
Hydrocarbon monoterpenes	55.54%	26.46%	46.70%
Hydrocarbon sesquiterpenes	4.94%	9.79%	7.10%
Oxygenated monoterpenes	35.78%	52.94%	41.94%
Alcohols	5.35%	12.42%	8.02%
Aldehydes	0.05%	0.35%	0.1%
Esters	3.68%	7.59%	5.98%
Oxides	24.32%	22.19%	24.67%
Ketones	0.05%	0.42%	0.07%
Phenolic compounds	2.33%	9.97%	3.1%
Oxygenated sesquiterpenes	1.84%	8.59%	2.52%
Alcohols	1.16%	4.38%	1.19%
Oxides	0.68%	4.21%	1.33%
Others oxygenated compounds	0.63%	0.54%	0.30%
Alcohols	0.05%	–	–
Aldehydes	0.3%	0.26	0.05
Esters	0.28%	0.28	0.15
Ketones	–	–	0.1
Total non-oxygenated compounds	60.48%	36.25	53.80
Total oxygenated compounds	38.25%	62.07%	44.76%
Total compounds	98.73%	98.32%	98.56%
Amount of extracted EO (g EO/100 g dry Matter)	0.51 ± 0.04	0.57 ± 0.12	0.61 ± 0.03

[a] HD-EO: Essential oils extracted by hydrodistillation of Algerian myrtle leaves dried to 16.2 g H_2O/100 g dry matter.
[b] DIC-EO: Essential oils directly extracted from Algerian myrtle leaves dried to 16.2 g H_2O/100 g dry matter, by DIC treatment at 0.6 MPa steam pressure, for 120 s as total thermal treatment time and with 4 cycles.
[c] HD-DIC-EO: Essential oil extracted by hydrodistillation of Algerian myrtle leaves dried to 16.2 g H_2O/100 g dry matter and treated by DIC at 0.2 MPa steam pressure, for 60 s as total thermal treatment time and with 3 cycles.

essential oils by DIC and this was obtained with the highest values of saturated steam pressure (0.5 or 0.6 MPa) and 4 or 5 cycles.

The highest value of residual essential oils corresponded to the least severe DIC treatment under the following conditions: 0.2 MPa, 60 s, 3 cycles. The quantity of residual oil was about 20% higher than with untreated raw material; this suggests that the DIC treatment resulted in a higher availability of essential oils, which was observed in some other cases and has been noted by various authors [39].

Table 2 summarizes the relative percentage of the constituent classes and families of the essential oils; 78 compounds were identified, representing 98.56% of the total chemical composition. All these essential oil components gave this strong and pleasant smell and were light yellow in color. Overall, the chemical composition was similar to that obtained from untreated myrtle with, however, some variations such as the increase in linalool from 1.32% to 2.73%, and α-terpineol from 2.51% to 3.62%. There was also a slight increase in α-Terpinyl acetate, methyl-eugenol, β-caryophyllene, α-humulene, β-selinene, α-selinene, and finally caryophyllene oxide, which increased from 0.85% to 1.11%, from 2.33% to 3%, from 0.88% to 1.39%, from 0.36% to 1.28%, from 0.76% to 1.07, from 0.75% to 1.15% and from 0.63% to 1.28%, respectively. Thymol (0.05%) and methyl citronellate (0.06%) were also detected but at very low concentrations. The monoterpene hydrocarbons and oxygenated monoterpenes were again predominant with 46.7% and 32.89%, respectively, of the total composition of the essential oils compared with 55.54% and 29.75% for untreated myrtle leaves. Fig. 4 highlights the comparative effects of hydrodistillation, direct DIC extraction and hydrodistillation on DIC-treated matter, in terms of the composition of oxygenated and non-oxygenated compounds.

The point that deserves mention is that the extraction kinetics in hydrodistillation are much faster when myrtle leaves are first treated with DIC than when untreated (Fig. 3). Indeed large droplets of essential oils began to appear almost instantly with
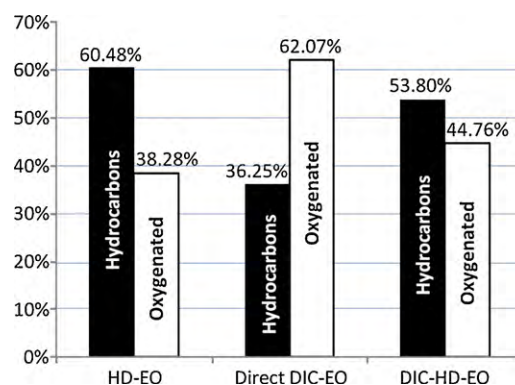

Fig. 4. Comparative composition of myrtle essential oils in terms of hydrocarbon and oxygenated compounds versus extraction processes (■: hydrocarbon compounds; □ oxygenated compounds).

the first drops of distillate, which was not the case with untreated plants.

This is probably due to the fact that the treatment causes structural changes in the plant resulting from a greater availability and a better initial accessibility of cells containing volatile compounds, coupled with the expansion and the appearance of alveoli in the solid matrix leading to an increase in porosity, which improves the diffusivity of the water within the plant.

3.2. Results with the response surface method (RSM)

In the experimental design, we used the direct DIC extraction (DIC-yield), the hydrodistillation yield after DIC treatment (HD-DIC-yield) and the total extraction (T-yield), (all expressed in g of essential oils/100 g of dry matter) as the response parameters (or dependent variables). Fig. 5 shows the effects of the operating parameters in terms of each response on a Pareto chart, which is usually introduced to describe a phenomenon in which 95% of the
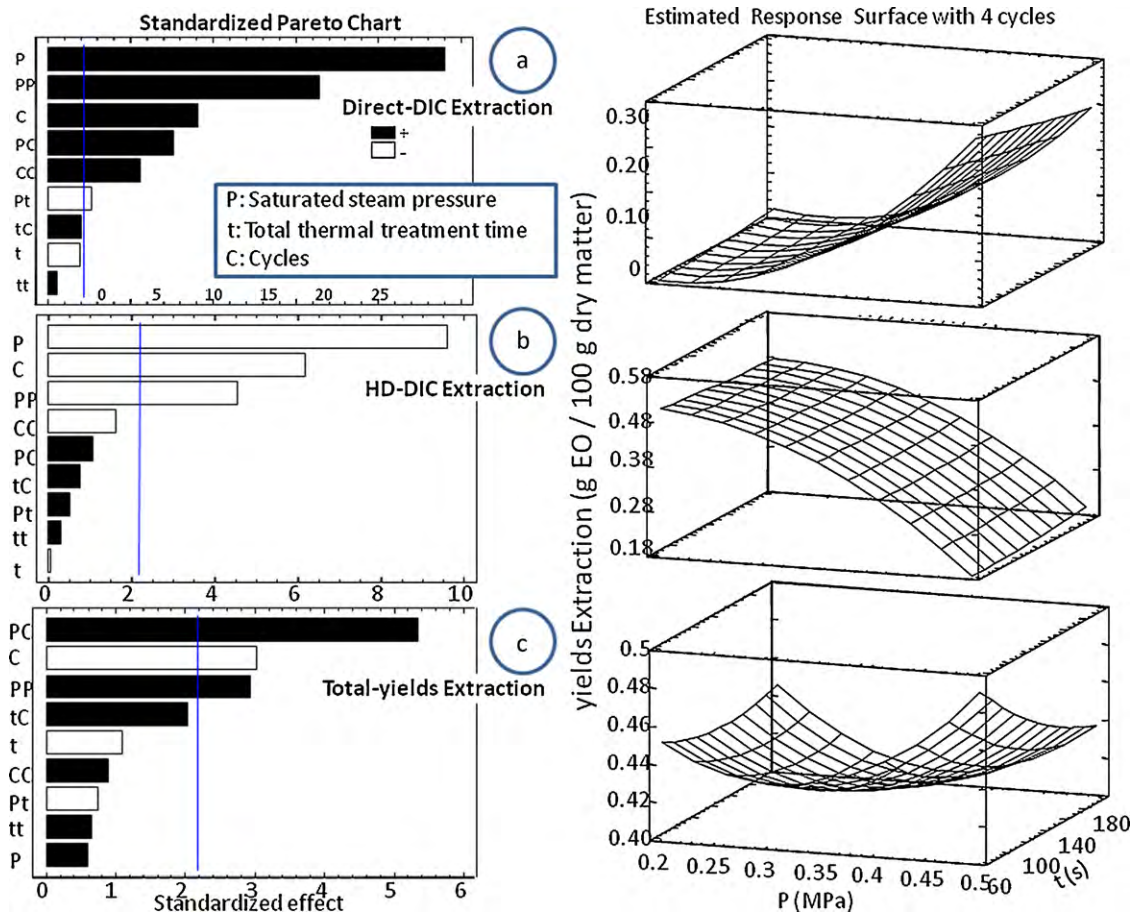
Fig. 5. Pareto chart and response surface of RSM experimental design by (a) direct extraction by DIC treatment, (b) hydrodistillation of myrtle leaves treated by DIC, and (c) total extraction yields after direct extraction by DIC with hydrodistillation of DIC-treated myrtle leaves from an experimental design, with steam pressure (P), total thermal treatment time (t), and number of cycles (C) as DIC operating parameters.

variation observed in experimental processes can be explained by a mere 5% of the causes of that variation.

In the case of direct DIC extraction, the effects of saturated steam pressure (P) showed that the processing temperature and the number of cycles (C) were the most relevant, whereas the total thermal treatment (t) had a very low impact. It is worth noting that the higher P and C, the higher the direct DIC-yields.

It was then possible to establish an empirical model of the DIC-yields versus the DIC processing parameters. The R^2 value

($R^2 = 98.84\%$) directly proved that the DIC treatment was a very relevant extraction process with essential oils:

$$DIC - yield = 1.18611 - 3.54999P - 0.000726108t - 0.403036C$$
$$+ 4.52199P^2 - 0.00251389Pt + 0.435833PC$$
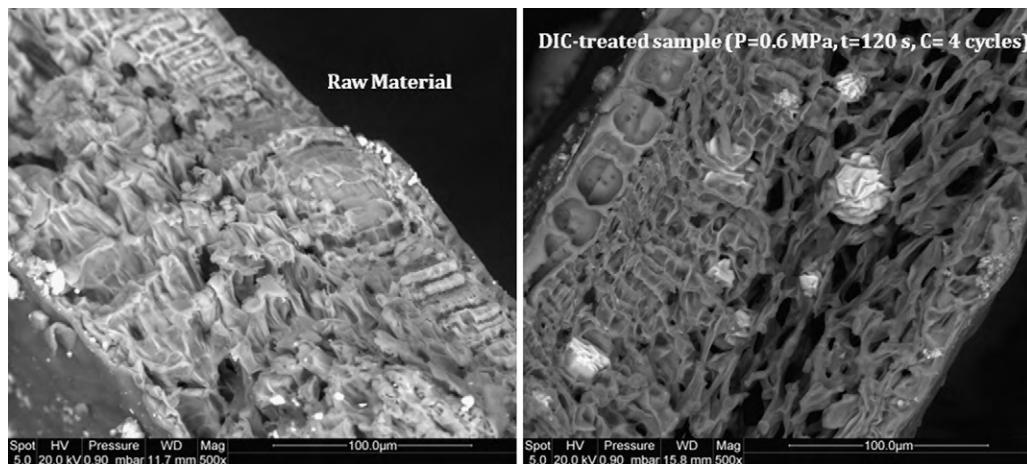$$+ 9.11153E - 7t^2 + 0.00029375tC + 0.034393C^2$$

Fig. 6. SEM microstructure of Algerian myrtle leaves: (a) raw material and (b) treated with instant controlled pressure drop at 0.6 MPa, 120 s, 4 cycles.

By noting that thermal treatment time had little or no impact on direct extraction yield, Besombes and co-workers (submitted) could assume that this operation was not primarily due to the evaporation of the molecules concerned. Thus, the process of direct extraction of essential oils using DIC mainly involved the phenomenon of the autovaporization of volatile compounds which quickly appear when the pressure drops instantaneously.

Furthermore, the increase in porosity and the appearance of holes due to the possible expansion of the matter allowed water and other volatile compounds to be easily expelled and then condense on the vacuum tank. Moreover, as this decompression was instantaneous, the structure of myrtle leaves was modified but the rupture of cell walls occurred only in high severity DIC treatment.

In the case of hydrodistillation carried out on DIC-treated myrtle leaves, the main effects were observed with these same parameters, P and C, and the effects of thermal treatment time were weak (See Pareto chart Fig. 5).

Finally, the total extracts of essential oil using direct DIC extraction (DIC-yields) and by hydrodistillation of DIC-treated material (HD-DIC-yields) was calculated in order to estimate more precisely the real initial concentration. Fig. 5 shows small variations of this parameter, probably due to the quantity of essential oils lost on the vacuum tank wall.

Here too, the values of DIC-yields were systematically underestimated because a quantity of essential oils retained on the vacuum tank wall or within the vacuum pump (light compounds). However, corroborating the conclusion of Allaf et al. [54], the results have proven the capacity of DIC to very quickly extract high yields of myrtle leaf essential oil, with lower energy consumption and using less steam and water.

3.3. Impact of treatments on microstructure of plant

It is well known that the DIC treatment can modify the structure of plant at various and controlled levels although this is highly dependent on the operating parameters. Such modifications from interstitial zone to broken cell walls, can greatly modify structural characteristics (porosity, specific surface area, etc.). It would be then possible to control functional and technological capacities that help to greatly intensify mass transfer phenomena by improving diffusivity and permeability within the plant. It is also possible to increase the availability and/or the initial accessibility of some high added-value compounds. In the case of myrtle leaves, the SEM images revealed structural differences between raw material and DIC-treated samples. Before DIC treatment, plants had a compact and relatively well organized cell structure. The impact of DIC treatment, which led to an evident expansion of the structure (Fig. 6) has kept the main part of the cells intact thus proving the great structural stability of the leaves.

4. Conclusion

The feasibility of using DIC for a direct extraction of essential oils from Algerian myrtle leaves was proved. It was possible to optimize the operation to get yields 10% higher than hydrodistillation, in a shorter time (2 min compared with 180 min) and of a better quality as revealed by the composition and antioxidant activity (non shown results). Compared with extraction by hydrodistillation, it is worth noting the lower energy and water consumption. This may be correlated with some expansion effects which, however, have no great impact in terms of the cell wall structure.

The analysis by GC-FID and GC–MS of DIC-EO, HD-EO, and HD-DIC-EO samples of essential oils allowed us to identify 78 compounds. Although α-pinene was the major component of myrtle leaves, whatever the extraction process (DIC, hydrodistillation, and DIC followed by hydrodistillation), DIC-EO was characterized by a slight increase in 1,8-cineol. It is also worth noting that DIC-EO was distinguished from the other two oils by a greater content of oxygenated compounds, which normally have great importance since, together with phenolic compounds, they contribute most to the flavor and aroma of an essential oil and ascribe its quality. There is also the advantage of the presence of phenolic compounds such as eugenol, which is recognized as a potent inhibitor of platelet aggression and for its local anesthetic and anti-inflammatory properties.

5. Nomenclature

C	the number of DIC cycles
D_{eff}	effective diffusivity of water within the solid medium ($m^2\,s^{-1}$)
DIC-EO	essential oil directly extracted using DIC (g EO/100 g dry matter)
HD-EO	essential oil extracted by hydrodistillation using raw material (g EO/100 g dry matter)
HD-DIC-EO	essential oil extracted by hydrodistillation applied on DIC-treated samples (g EO/100 g dry matter)
P	saturated steam pressure in DIC treatment (MPa)
t	total thermal treatment time (s)

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