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## ARTICLE INFO

## ABSTRACT

New process, instantaneous controlled pressure drop (DIC) was applied on *Cananga odorata* dry flowers with the aim to isolate essential oil. DIC is based on high temperature, short time heating followed by an abrupt pressure drop into a vacuum. A part of volatile compounds is carried away from flowers in the form of vapor (*DIC direct oil*) that evolves adiabatically during the pressure drop (*proper isolation process*) and the other part remains in the DIC-treated flowers (*DIC residual oil*). In the present paper, the effect of DIC cycle number (1–9) and heating time (4.3–15.7 min) on the availability of oil compounds was investigated at three levels of steam pressure (0.28, 0.4 and 0.6 MPa). The availability was defined as the amount of a compound in direct or residual oil divided by the amount of this compound in the reference oil extracted from non-treated flowers by chloroform during 2 h. The total availability and yield of volatiles in the direct oil increased with pressure and cycle number. At a higher pressure, the effect of heating time was insignificant. The amount of oxygenated monoterpenes and other light oxygenated compounds (i.e. predominantly exogenous compounds) in the residual flowers was lower than in the direct oil and this amount decreased with cycle number. On the other hand, the availability of oxygenated sesquiterpenes and other heavy oxygenated compounds (i.e. predominantly endogenous compounds) in residual flowers exhibited a maximum for about five cycles and their quantity at this point was three times as much as in the direct oil. The total availability of each compound at 0.6 MPa was higher than one. The rapid DIC process (0.6 MPa, 8 cycles, 6 min) gave better results than steam distillation (16 h) concerning direct oil yield (2.8% dm versus 2.5% dm) and content of oxygenated compounds (72.5% versus 61.7%).

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## 1. Introduction

Besides its long utilisation as fragrance component in perfumery, the application of cananga or ylang–ylang essential oils as flavoring agent in candies, icings, frozen dairy, pudding, baked goods, soft drinks and chewing gums have increased in the recent years [1–4]. Cananga refers to plant species of *Cananga odorata forma macrophylla* whereas ylang–ylang is belonging to *forma genuina*. These oils have been approved as food additives by US Food and Drug Administration (FDA) and have been determined to be safe (GRAS) for food uses by Flavor and Extract Manufacturers Association (FEMA) and the International Organisation of Flavor Industries (IOFIs) [5,6]. Burdock et al. [5] reported that the present consumption level of cananga or ylang oil (0.1 μg/(kg day), which is estimated using per capita daily intake method) from food flavoring does not pose human health effects.

The cananga and ylang oils are mostly obtained by steam distillation of fresh mature flowers and rarely by solvent extraction [1,4]. Losses of some volatile compounds, long processing time, low-isolation efficiency, compounds degradation and toxic solvent residue in the extract may be encountered using these conventional essential oil isolation methods [7,8]. The modern process such as supercritical fluid extraction, mainly using carbon dioxide, allows us to obtain a high-quality extract, but the high-fixed cost limits its application to high-added value products [9,10]. Moreover, this process is less convenient for polar compounds and it extracts also culticular waxes and lipids [11,12]. The green technologies for extraction of essential oil from aromatic plants, such as vacuum microwave hydrodistillation (VMHD) and solvent-free microwave hydrodistillation (SFME), have been successfully developed [13–19]. They consist in combination of microwave heating and dry distillation, performed at atmospheric pressure (SFME), and followed by slow pressure drop rate into vacuum (VMHD), without added any solvent or water. These processes yielded the better quality extracts (more oxygenated compounds) with faster kinetics extraction than the conventional ones. However, Klima [20] reported the presence of non-uniformity of material temperature due to non-homogeneity of electromagnetic field during microwave heating.

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A new isolation process, instantaneous controlled pressure drop (DIC) [21], allows us to obtain a high-quality essential oil in a short time. DIC process is based on the thermo-mechanical effects induced by subjecting the raw material for a short period of time to saturated steam (about 0.1–0.6 MPa according to the product), followed by an abrupt pressure drop towards a vacuum (about 5 kPa). This abrupt pressure drop ($\Delta P/\Delta t > 0.5$ MPa/s) provokes simultaneously auto-vaporization of volatile compounds, instantaneous cooling of the products which stops thermal degradation, and swelling and rupture of the cell walls. The created porous structure then enhances mass transfer.

In this work, the effect of steam pressure, cycle number and heating time of DIC process on the amount of essential oil compounds in the residual flowers (*C. odorata f. macrophylla*) was studied using the response surface methodology (RSM). This study was completed by quantification of oil compounds in the DIC condensate at selected experimental conditions. The cananga oil compounds were grouped into light oxygenated compounds (LOCs), heavy oxygenated compounds (HOCs) and sesquiterpenes hydrocarbons (Ss). LOC consists of oxygenated monoterpenes (OMs) and other light oxygenated compounds (OLs) whereas HOC comprises oxygenated sesquiterpenes (OSs) and other heavy oxygenated compounds (OHs).

## 2. Experimental

The investigation of the effect of the DIC cycle number, time and pressure was carried out according to the protocol shown in Fig. 1. The investigation was done in two stages. First, the range of DIC parameters which gives a reasonable DIC isolation efficiency of cananga oil was identified. In order to economize experimental effort, the RSM analysis based on the essential oil availability in the DIC-treated flowers was applied [22]. This residual availability denotes the ratio of oil yield in the DIC-treated (residual oil) and non-treated plants (reference oil). Using the assumption

that the sum of the volatiles in residual oil and DIC condensate is equal to the quantity in reference oil, the amount of oil which is isolated in form of vapor (DIC isolation efficiency) can be estimated. The effect of DIC treatment on the improvement of solvent extraction of non-volatiles compounds was also studied. In this case, the response of RSM analysis was the yield of DIC residual extract. The DIC cycle number (1–9) and heating time (4.3–15.7 min) were varied to build an experimental design at three pressure levels (0.28, 0.4 and 0.6 MPa). The last stage was the quantification of volatiles in DIC condensate at the selected range of parameters resulted from the above-described RSM analysis. The sum of the volatiles in DIC condensate and DIC-treated flowers was compared with the amount in the non-treated flowers.

In order to isolate the residual and reference oils and extracts, chloroform extraction during 2 h was applied on the DIC-treated and non-treated flowers. The risk of thermal degradation can be reduced using solvent extraction rather than steam distillation. During 2 h, chloroform extracted from non-treated flowers 63% of the volatiles and 78% of the extract obtained by the same solvent in 8 h [23]. The solvent extraction technique was described elsewhere [23,24]. The flower structure before and after DIC treatment was investigated by scanning electron microscopy (Jeol 5410 LV SEM).

### 2.1. Materials

Fully mature cananga flowers (*C. odorata f. macrophylla*) were picked up at blossom in Lawang, East Java, Indonesia. The flowers were air-dried to a final moisture content of 10.2% dry matter (dm) and stored at room temperature prior to use.

External standard solutions at 0.2, 0.6, 1.2, 2, 3, 5 mg/mL of methyl nonadecanoate (puriss. grade, Sigma–Aldrich Corp., Germany) in chloroform (GC grade, Carlo Erba Reagenti, France) were prepared for GC/MS analysis. NaCl (purum grade, Sigma–Aldrich Corp.), anhydrous $Na_2SO_4$ (for analysis, purity >99.5%, Fischer Chemicals AG), and chloroform (GC grade) were used as demul-
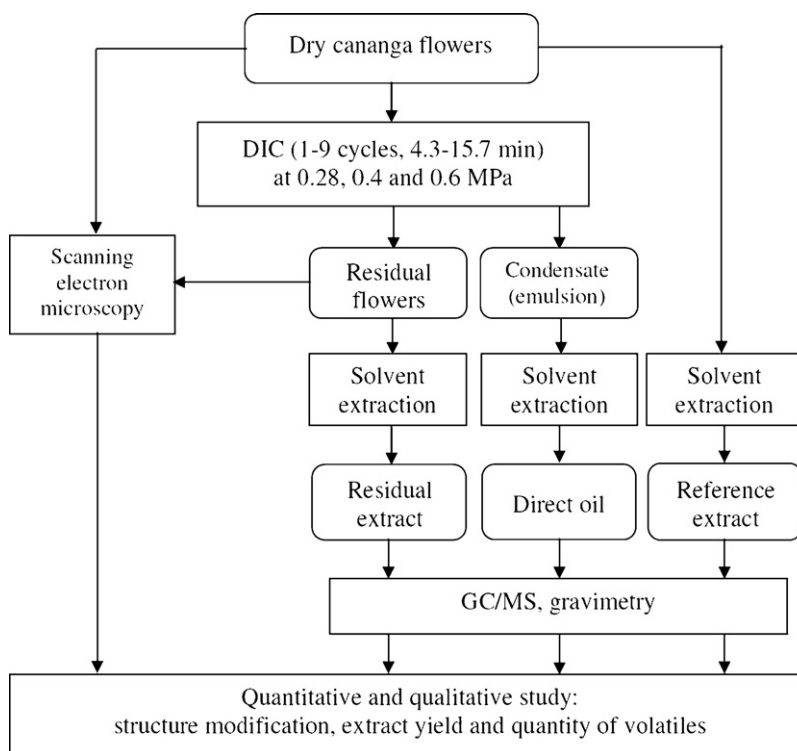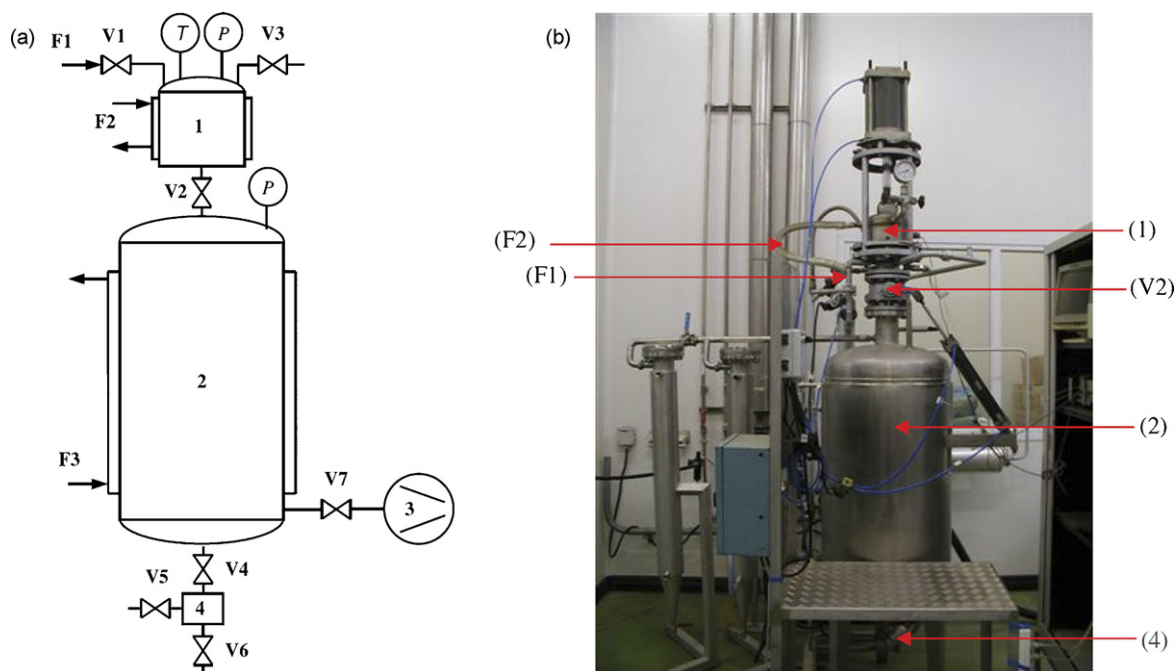


**Fig. 1.** Experimental protocol.

**Fig. 2.** Instantaneous controlled pressure drop apparatus. (a) Schematic diagram: (1) autoclave with heating jacket; (V2) rapid valve; (2) vacuum tank with cooling water jacket; (3) vacuum pump; (4) extract container; F1 and F2, steam flow; F3, cooling water flow. (b) Photo.

sifier, drying agent and solvent, respectively, in the liquid–liquid extraction step of DIC condensate; the latter was also used in the solvent extraction of untreated and residual flowers.

### 2.2. Separation methods

#### 2.2.1. DIC apparatus and procedure

The schematic diagram and the photo of the DIC apparatus are shown in Fig. 2. Dry cananga flowers (40 g dm) were treated in the 6 L autoclave (1) equipped with a double jacket, thermocouples and a pressure gauge. Steam used for flower heating was injected through the valve (V1). The autoclave was connected via 80 mm spherical valve (V2) to a vacuum tank (2) with a volume of 285 L. The valve was controlled by a rapid pneumatic actuator. The vacuum tank was cooled through a double jacket for condensation of the vapor, evolved by vaporization of water and volatile molecules during pressure drop. Condensate was recuperated through a trap (4). A water ring pump (3) maintained the tank pressure at about 5 kPa. The atmospheric pressure in the autoclave could be installed by opening a vent (V3).

Temperature and pressure histories of one DIC cycle are shown in Fig. 3. At the beginning, the autoclave is at the atmospheric pressure (a). After opening the spherical valve, a vacuum about 5 kPa is installed in the autoclave (b). The initial vacuum allows better penetration of the heating fluid which enhances the heat transfer in flowers. After closing this valve, saturated steam is injected into the autoclave (c) and maintained at a fixed pressure level for a predetermined time (d). At this period, the temperature in the autoclave corresponds to the temperature of the saturated steam at the pressure in the autoclave. After this thermal treatment the steam is cut-off and the spherical valve is opened rapidly (in less than 0.2 s) which results in an abrupt pressure drop in the autoclave (e). After the vacuum period (f), the spherical valve is closed and the atmospheric pressure installed (g).

In the multi-cycle DIC process (see Fig. 4), steam is injected again after the stage (f) and the pressure is controlled during the time (d). It means that $n$ cycles contain $n$ repetition of the stages (c)–(f). The
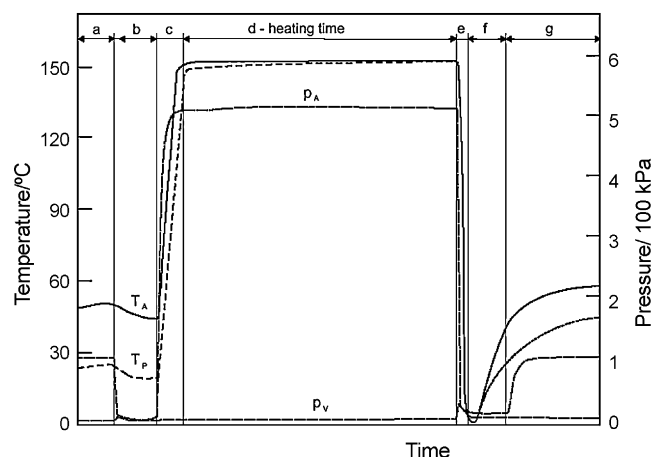


**Fig. 3.** Temperature and pressure history of a DIC processing cycle. $p_A$ pressure in autoclave, $p_V$ pressure in vacuum tank, $T_A$ temperature in autoclave, $T_P$ temperature of product: (a) sample at atmospheric pressure; (b) initial vacuum; (c) saturated steam injection to reach the selected pressure; (d) constant temperature corresponding to saturated steam pressure; (e) abrupt pressure drop towards vacuum; (f) vacuum; (g) releasing to the atmospheric pressure.
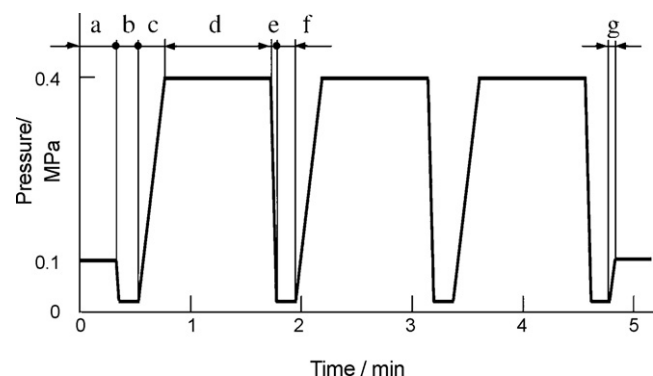


**Fig. 4.** Pressure history in multi-cycle DIC process.

last cycle is terminated by the stage (g). The total heating time is the heating time of all cycles ($n \times d$). All results given in this work concern the total heating time. The total processing time is a little longer due to the short stages (c), (e) and (f).

During the abrupt pressure drop, an adiabatic vaporization of the superheated water and volatile compounds takes place in the flowers. The vapor engenders mechanical stresses within the flowers. The blowing and breaking of cells depend on these stresses and viscoelastic behavior of the flower structure. It is a function of moisture content, temperature and heating time. The auto-vaporization as an adiabatic transformation induces also instantaneous cooling of the residual flowers (see Fig. 3). The exposure of product to a high temperature is limited to a short heating period which diminishes thermal degradation.

After the DIC treatment the flowers and the condensate were recuperated. The condensate, which was in the form of a very stable oil-in-water emulsion with droplet diameter less than 0.5 μm, was subjected to liquid–liquid extraction step with the aim to isolate volatiles. The flowers were subjected to residual analysis as described in Fig. 1.

### 2.2.2. Separation of volatiles from DIC condensate

In order to isolate volatiles, the DIC condensate was extracted three times using a separator funnel by the addition of chloroform

**Table 1**
Independent variables used in response surface methodology at a fixed pressure

| | Coded level | | | | |
|---|---|---|---|---|---|
| | $-\alpha$ | $-1$ | $0$ | $1$ | $+\alpha$ |
| Cycle number, $X_1$ | 1[a] | 2 | 5 | 8 | 9[a] |
| Heating time (min), $X_2$ | 4.3 | 6 | 10 | 14 | 15.7 |

$\alpha$ (coded axial distance of extreme point) $= \sqrt[4]{2^N}$, where $N$ is the number of independent variables. In our case $\alpha = 1.4142$.

[a] The cycle numbers 0.76 and 9.3 were rounded on 1 and 9, respectively.

and NaCl to facilitate the emulsion breakdown. Sodium sulphate was added to remove moisture and the organic phase was then concentrated under a vacuum in a rotary-evaporator at 30 °C. Each dried sample was diluted with chloroform to 20 mL and stored in the dark at 4 °C prior to yield and composition analysis by GC/MS.

### 2.3. Methods of analysis

#### 2.3.1. Yield and availability

The yield of reference and residual extracts ($y_{ef}$ and $y_{es}$) is defined as the ratio of the extract mass ($m_{ef}$ or $m_{es}$) to the dry matter
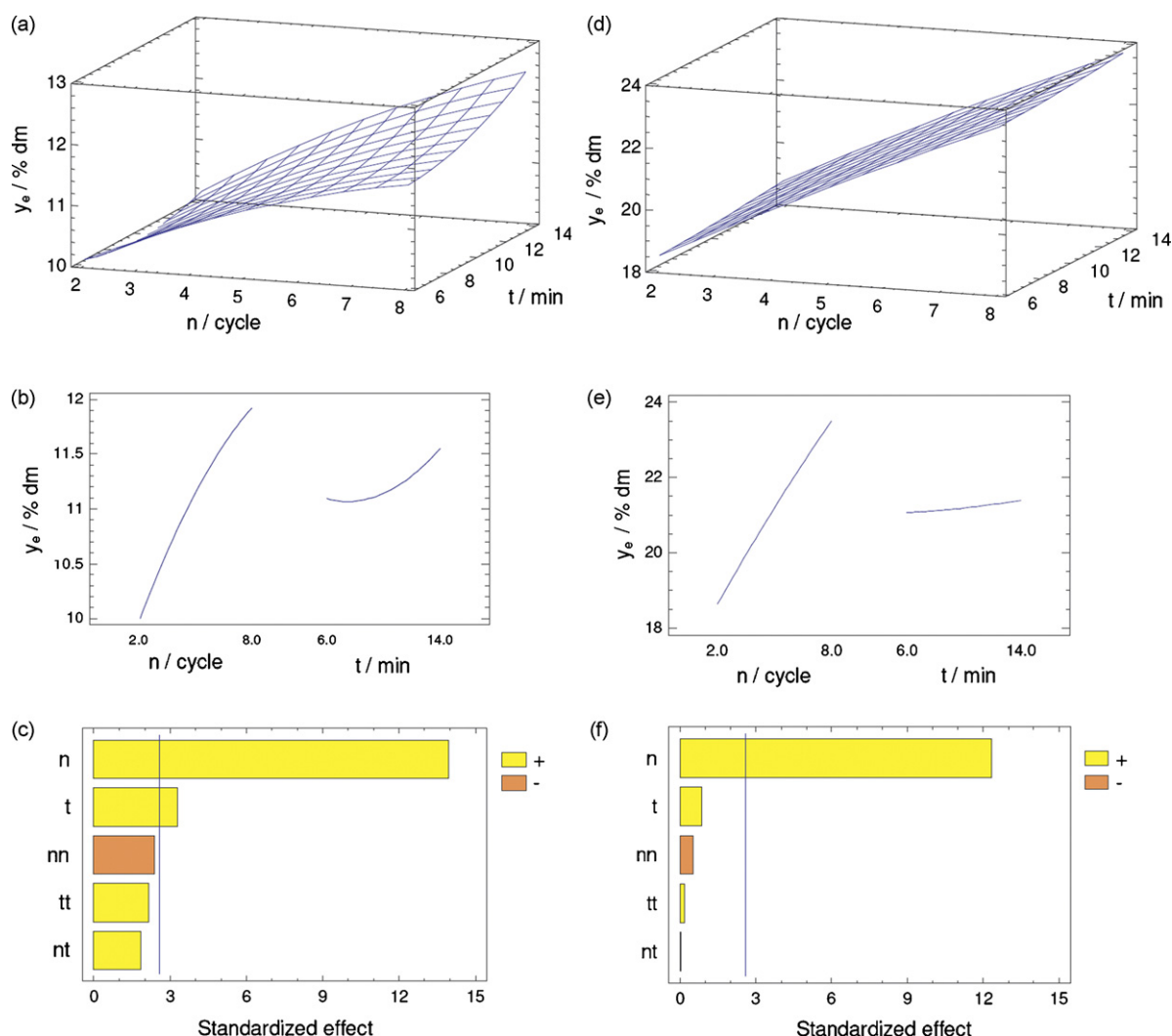


**Fig. 5.** Effect of cycle number and heating time on DIC residual extract yield at 0.28 MPa (left) and 0.6 MPa (right): (a and d) response surfaces; (b and e) main effects; (c and f) pareto charts.

**Table 2**
Regression coefficients and optimal values for DIC residual extract yield

|  | 0.28 MPa | 0.4 MPa | 0.6 MPa |
|---|---|---|---|
| $\beta_0$ | 10.2655 | 12.3271 | 16.6055 |
| $\beta_1$ | 0.3977 | 0.9325 | 0.9518 |
| $\beta_2$ | −0.2446 | 0.0055 | −0.0059 |
| $\beta_{11}$ | −0.0221 | −0.0260 | −0.0136 |
| $\beta_{12}$ | 0.0151 | −0.0092 | −0.0006 |
| $\beta_{22}$ | 0.0113 | 0.0060 | 0.0025 |
| $R^2$ (%) | 97.8 | 98.7 | 96.8 |
| Maximum |  |  |  |
| $y_e$ (% dm) | 13.2 | 19.0 | 24.7 |
| $n$ (cycle) | 9 | 9 | 9 |
| $t$ (min) | 15.7 | 15.7 | 15.7 |

of flowers ($m_{dm}$):

$$y_{ef} = \frac{m_{ef}}{m_{dm}}, \tag{1}$$

$$y_{es} = \frac{m_{es}}{m_{dm}}. \tag{2}$$

Extracts were obtained either from the DIC-treated flowers (residual extract) or from untreated flowers (reference extract) using chloroform during 2 h. The mass of extract is its total mass comprising volatile compounds and non-volatile molecules (resin, wax, fatty oil, colorant, etc.). It was measured gravimetrically.

The yield of reference oil ($y_{of}$) and DIC direct and residual oils ($y_{od}$ and $y_{os}$) is based on dry matter of flowers:

$$y_{of} = \frac{m_{of}}{m_{dm}}, \quad y_{od} = \frac{m_{od}}{m_{dm}}, \tag{3}$$

$$y_{os} = \frac{m_{os}}{m_{dm}}, \tag{4}$$

where the oil mass was computed from GC peak area of all volatiles molecules using the external standard method with methyl non-adecanoate. DIC direct oil is composed of the volatiles molecules isolated from the DIC condensate. Reference oil contains the volatile molecules extracted from non-treated dry flowers by chloroform during 2 h. DIC residual oil means the volatile molecules isolated from DIC-treated flowers using the same solvent and time.

The yield of the compound $j$ is calculated as

$$y_{jf} = y_{of}x_{jf}, \quad y_{jd} = y_{od}x_{jd}, \tag{5}$$

$$y_{js} = y_{os}x_{js}, \tag{6}$$

where $x_{jf}$, $x_{jd}$ and $x_{js}$ are the mass fraction in the reference, DIC direct and residual oils determined by GC/MS.
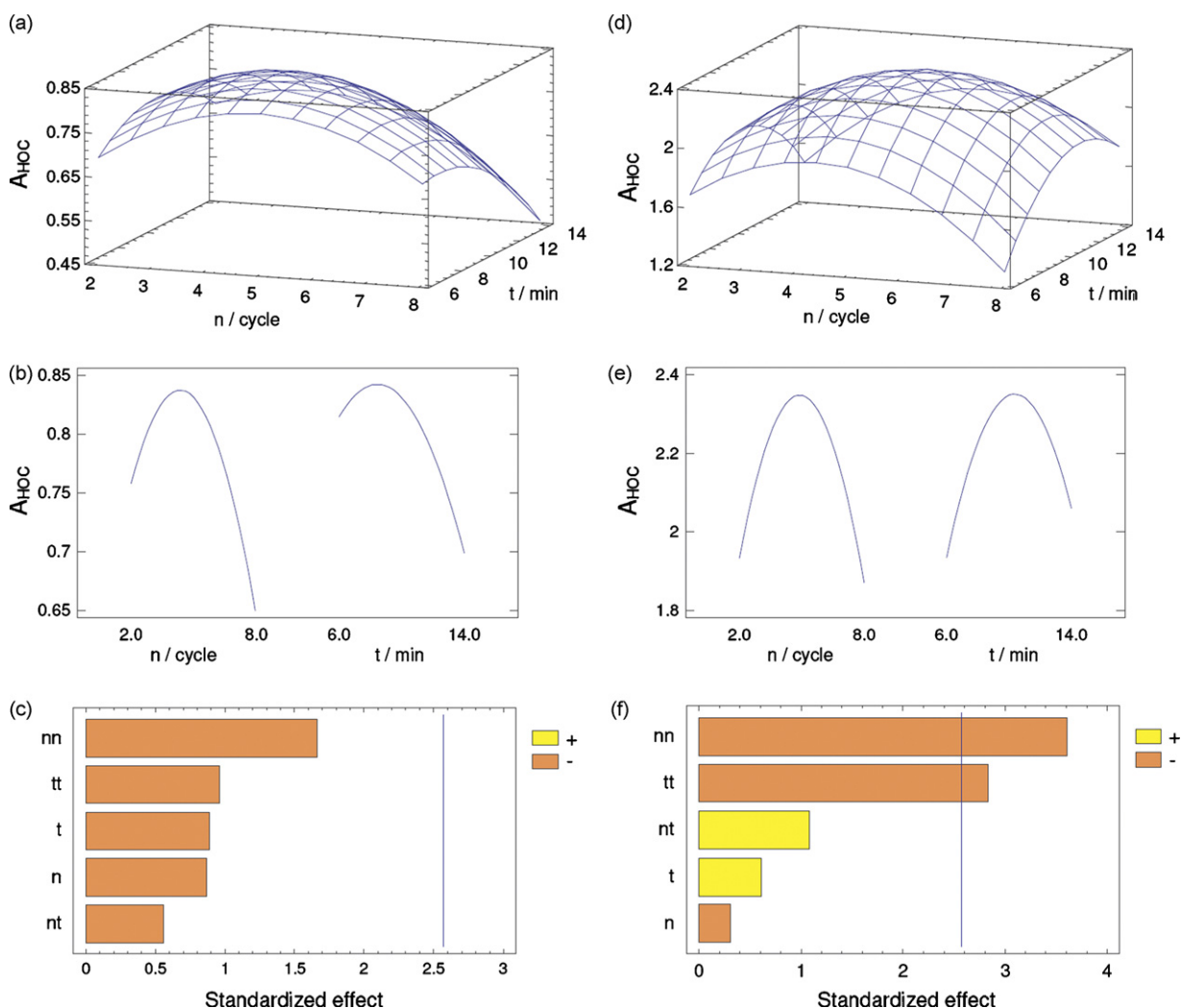


**Fig. 6.** Effect of cycle number and heating time on HOC availability in residual flowers at 0.28 MPa (left) and 0.6 MPa (right): (a and d) response surfaces; (b and e) main effects; (c and f) pareto charts. HOC: oxygenated sesquiterpenes and other heavy oxygenated compounds.

**Table 3**
Regression coefficients and optimal values for grouped compound availabilities in residual flowers

| | HOC (oxygenated sesquiterpenes and other heavy oxygenated compounds) | | | Sesquiterpenes hydrocarbons | | | LOC (oxygenated monoterpenes and other light oxygenated compounds) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.28 MPa | 0.4 MPa | 0.6 MPa | 0.28 MPa | 0.4 MPa | 0.6 MPa | 0.28 MPa | 0.4 MPa | 0.6 MPa |
| $\beta_0$ | 0.0306 | 0.9615 | −0.5286 | 0.3801 | 0.5277 | 0.5392 | 0.3324 | 0.7949 | 0.5043 |
| $\beta_1$ | 0.1679 | 0.2053 | 0.3535 | 0.0066 | −0.0428 | −0.051 | −0.0232 | −0.0817 | −0.0571 |
| $\beta_2$ | 0.1001 | −0.0741 | 0.388 | −0.0239 | −0.0153 | 0.0399 | −0.0246 | −0.043 | 0.0026 |
| $\beta_{11}$ | −0.0144 | −0.0221 | −0.0496 | 0.0003 | 0.0046 | 0.0033 | 0.0004 | 0.0026 | 0.0002 |
| $\beta_{21}$ | −0.0043 | 0.0002 | 0.0132 | −0.0017 | −0.0015 | −0.0009 | 0.0011 | 0.0015 | 0.0018 |
| $\beta_{22}$ | −0.0047 | 0.0038 | −0.0219 | 0.0014 | 0.001 | −0.002 | 0.0006 | 0.0013 | −0.0007 |
| $R^2$ (%) | 49 | 65 | 78.2 | 80.1 | 35.2 | 75.6 | 90.1 | 97.7 | 83.9 |
| Maximum | | | | | | | | | |
| $A$ | 0.85 | 1.24 | 2.35 | 0.35 | 0.49 | 0.7 | 0.22 | 0.58 | 0.46 |
| $n$ (cycle) | 5 | 5 | 5 | 1 | 1 | 1 | 1 | 1 | 1 |
| $t$ (min) | 8.6 | 15.7 | 10.3 | 15.7 | 15.7 | 9.9 | 4.3 | 4.3 | 4.3 |

The availability of the compound $j$ in the residual flowers (residual availability) is defined by the following expression:

$$A_j = \frac{y_{js}}{y_{jf}}. \tag{7}$$

The total availability of the compound $j$ in the flowers is calculated from the sum of the yield of compound $j$ in DIC direct and residual oils:

$$A_{Tj} = \frac{y_{jd} + y_{js}}{y_{jf}}, \tag{8}$$
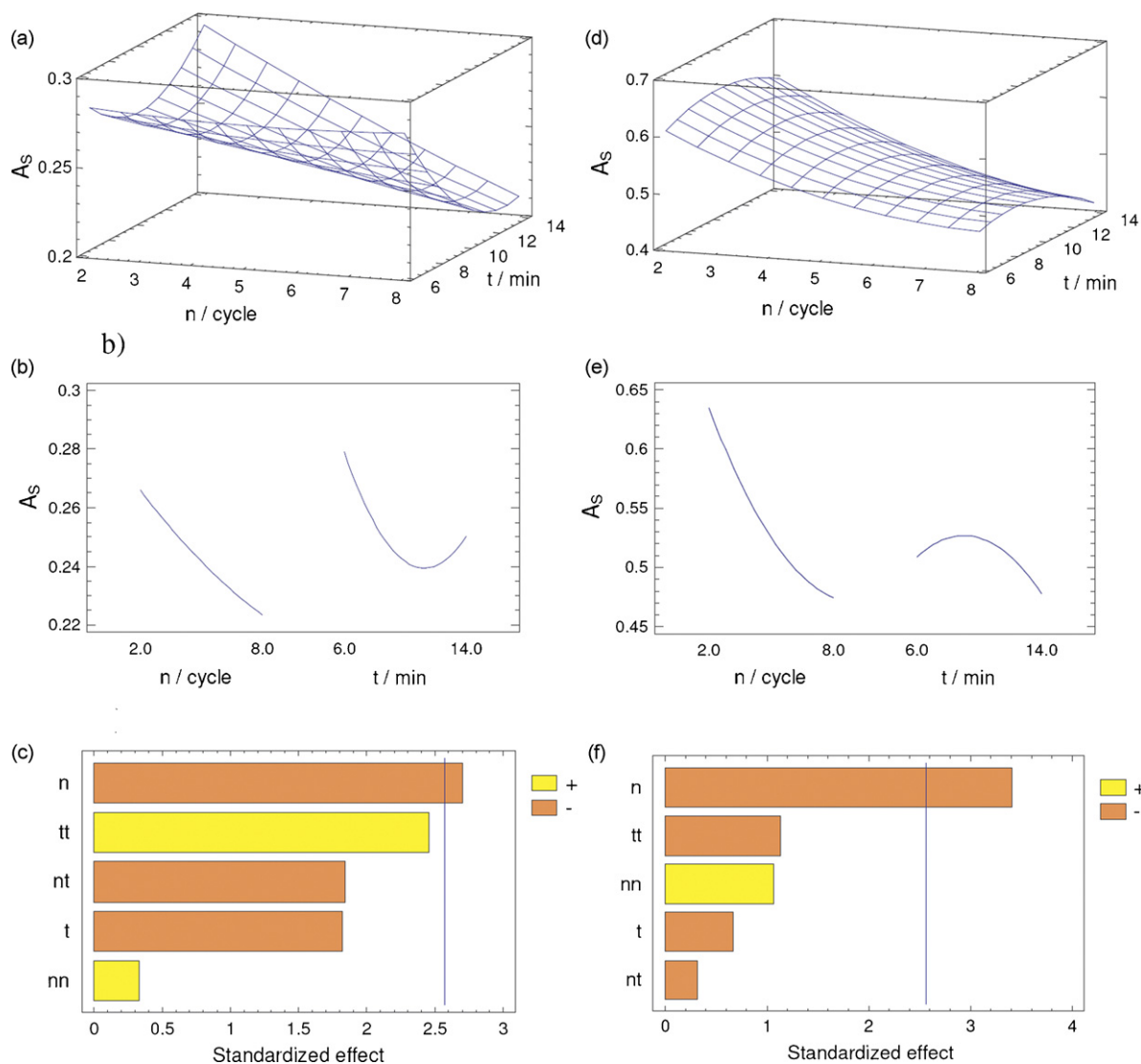


**Fig. 7.** Effect of cycle number and heating time on sesquiterpenes hydrocarbons availability in residual flowers at 0.28 MPa (left) and 0.6 MPa (right): (a and d) response surfaces; (b and e) main effects; (c and f) pareto charts.

where $y_{jf}$ is the yield of compound $j$ in reference oil obtained by 2 h chloroform extraction.

Under the assumption that the DIC treatment only isolates a part of a compound and does not increase its availability in residual flowers ($A_{Tj} = 1$), the DIC isolation efficiency can be estimated as

$$E_j = 1 - A_j. \tag{9}$$

It should be noted that the availability in residual flowers was often increased by the DIC treatment which led to underestimation of $E_j$.

### 2.3.2. Experimental design

Response surface methodology was used for the investigation of the effect of DIC cycle number and heating time on the DIC residual extract yield and the availability of three volatile compound groups in residual flowers: sesquiterpenes hydrocarbons and oxygenated compounds such as oxygenated monoterpenes and other LOCs and oxygenated sesquiterpenes and other HOCs. The experimental design was elaborated for three levels of pressure (0.28, 0.4 and 0.6 MPa). The cycle number (1–9) and heating time (4.3–15.7 min) were varied to build a five-level central composite design (CCD) [25].

A second-order polynomial model was used to express the responses as a function of independent variables:

$$\eta = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \beta_{12} X_1 X_2, \tag{10}$$

where $\eta$ is the response, $\beta_0$, $\beta_i$, $\beta_{ii}$ and $\beta_{12}$ are the regression coefficients and $X_i$ and $X_m$ are the independent variables with actual value in original units.

Based on predicted model equations, surface plots were generated by Statgraphics Plus 5.1 software. The contour plots for dependent variables were superimposed to find out the optimum combinations of independent variables. Analyses of variance (ANOVA) were performed to determine significant differences between independent variables ($P \leq 0.05$).

For 2 variables, the central composite design yielded 11 experiments with 4 factorials points, 4 extra points (star points) and 3 central points for replications. The experiments were run in random in order to minimize the effects of unexpected variability in the observed responses due to extraneous factors. The coded and uncoded independent variables are shown in Table 1.
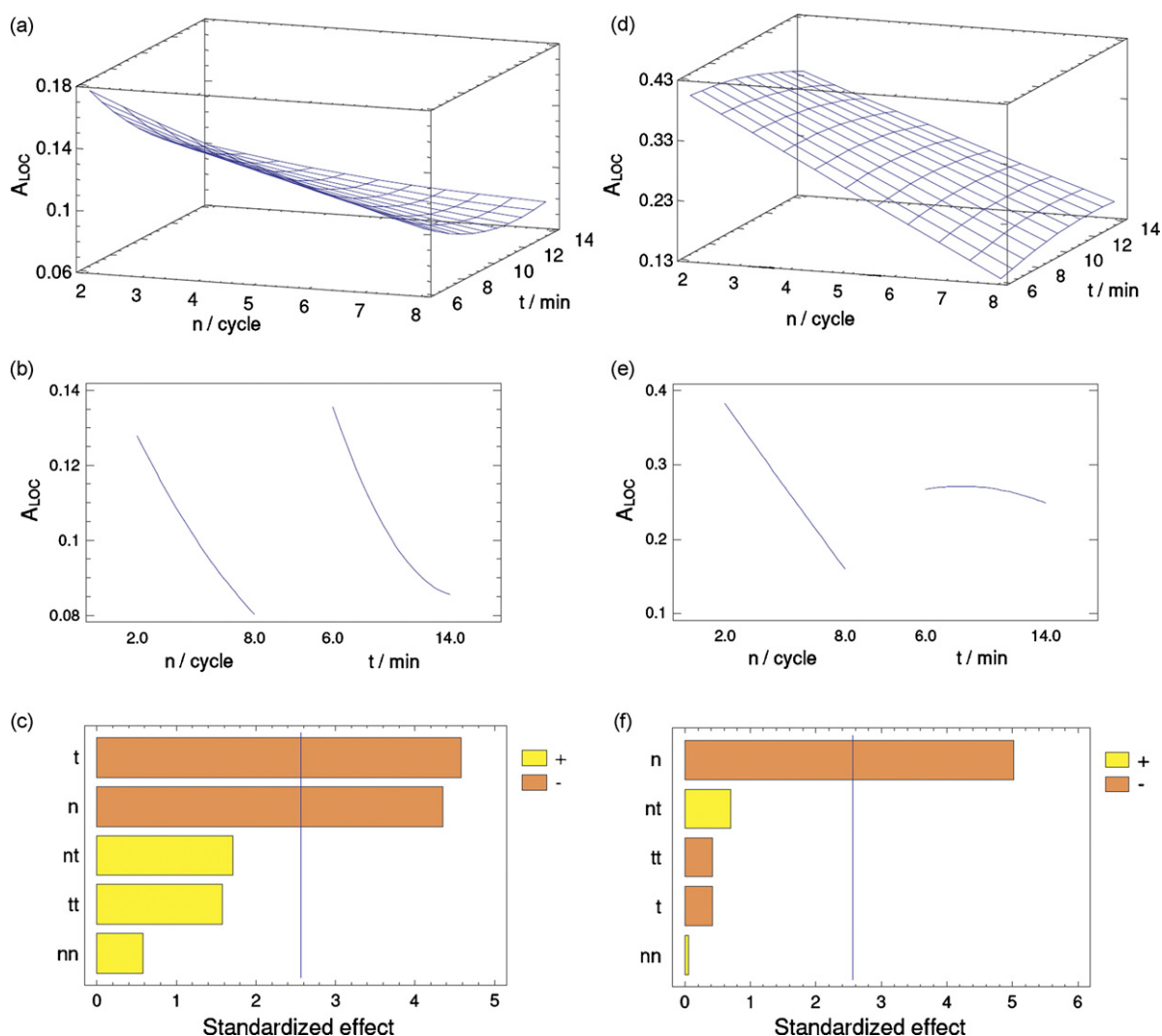


**Fig. 8.** Effect of cycle number and heating time on LOC availability in residual flowers at 0.28 MPa (left) and 0.6 MPa (right): (a and d) response surfaces; (b and e) main effects; (c and f) pareto charts. LOC: oxygenated monoterpenes and other light oxygenated compounds.
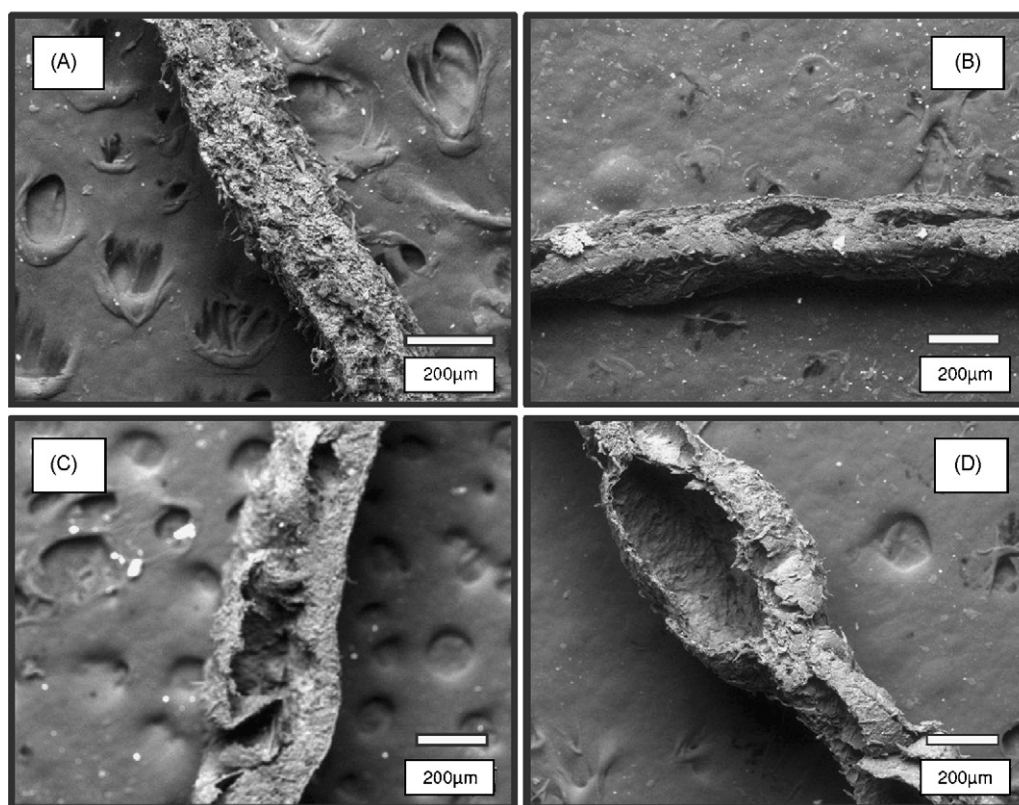
**Fig. 9.** Micrographs of untreated flowers (A) and DIC-treated flowers at 0.6 MPa, 2 cycles, 6 min (B); 0.4 MPa, 5 cycles, 4.4 min (C); 0.6 MPa, 5 cycles, 4.4 min (D).

### 2.3.3. Gas chromatography/mass spectrometry

The volatile molecules were analyzed by gas chromatography coupled to mass spectrometry (GC/MS). We used this method for a comparative study of DIC isolation and steam distillation even if GC/FID is widely used for quantitative analysis of essential oil. A Varian computerized system comprising a 3900 gas chromatograph equipped with a fused-silica-capillary column with a non-polar stationary phase poly (dimethylsiloxane) CP-Sil 8 ($30 \, \text{m} \times 0.25 \, \text{mm} \times 0.25 \, \mu\text{m}$ film thickness) was connected to a 2100T mass spectrometer. The measurements were performed under the following conditions: carrier gas He; flow rate 1 mL/min; split 1:100; injection volume 0.1 μL; injection temperature 250 °C; the oven temperature was increased from 60 to 170 °C at 2.5 °C/min, from 170 to 250 °C at 10 °C/min and held at 250 °C for 5 min. The ionization mode was an electronic impact at 70 eV. Mass spectra and reconstructed chromatograms were obtained by automatic scanning in the mass range m/z 30–400 amu at 2.2 scans/s. Identification of the components was achieved by a comparison of their retention indices relative to C8–C32 $n$-alkanes (RI) and mass spectral fragmentation patterns with those found in the literature [26–29], and stored in the data bank (Varian NIST MS Database 1998 and Saturn libraries).

A quantitative analysis was performed by the method of peak area normalization without application of response factor corrections. Because of non-availability of authentic standards, all relative response factors were taken as one [30,31]. Individual calibration of each constituent with its authentic compounds as external standards can be easily done with simple oils, but it is impossible with more complex oils containing many interesting constituents such as cananga oil. Thus, in the present work, the quantity of essential oil and their principal components was determined by the external standard method using only one authentic compound, i.e. methyl nonadecanoate. The standard curves were generated by analysis of known concentrations of methyl nonadecanoate dissolved in chloroform. Then the quantification was made with linear calibration curve of the external standard and dilution factor.

## 3. Results and discussion

### 3.1. Analysis of residual flowers

The effect of cycle number and heating time on the DIC residual extract yield is shown in Fig. 5 for the extreme values of pressure (0.28 and 0.6 MPa). The residual extract was obtained by chloroform extraction (2 h) of the DIC-treated flowers. It consists of volatile compounds (oil) and non-volatile molecules such as resin, wax, pigment, fatty oil, etc. The residual extract yield is higher than the reference one and increases with increasing pressure and number of DIC cycles. At 0.6 MPa, 9 cycles ($t = 15.7$ min) result in a value of 24.7% dm, while 1 cycle gives a yield of 17.5% dm. At a higher pressure the heating time is insignificant parameter. The parameters of response surface for all three pressures are given in Table 2. Eq. (10) describes well the experimental data, $R^2 \geq 96.8\%$. The effect of the DIC parameters on the extract yield can be explained as follows. The abrupt pressure drop provokes adiabatic vaporization of water and volatile molecules within the flowers. This auto-vaporization

**Table 4**
Yield and grouped compounds of volatiles in the reference oil (non-treated flowers), DIC direct and residual oils (0.6 MPa, 8 cycles, 6 min)

| Compounds | DIC direct oil | DIC residual oil | Reference oil |
|---|---|---|---|
| LOC (%) | 15.0 | 0.5 | 5.6 |
| S (%) | 25.9 | 19.9 | 38.7 |
| HOC (%) | 57.5 | 79 | 55.4 |
| Oil yield (% dm) | 2.77 | 2.8 | 3.1 |

induces swelling and alveolation of the flower structure. With increasing number of pressure drops (i.e. number of DIC cycles), the alveolation effect also increases. More deep and large canals are formed. The steam penetrates more easily during next cycle and heats the flowers more rapidly. Thus the rheological state of material (viscoelasticity) necessary for alveolation is achieved in a shorter time. The mechanical constraints depend on the amount of vapor produced by auto-vaporization. This amount is proportional to the temperature drop, which increases with pressure. In the case

of saturated steam, the pressure defines the treatment temperature. Finally, it can be concluded that the higher the steam pressure, the more complex the alveolation, then the shorter the heating time is necessary. It is the reason why the heating time becomes less significant with increasing pressure. The mass transfer in the resulting porous structure with broken cells is enhanced and the solvent penetration and extraction is more efficient. These results demonstrate the impact of DIC treatment on the improvement of separation of non-volatile molecules.
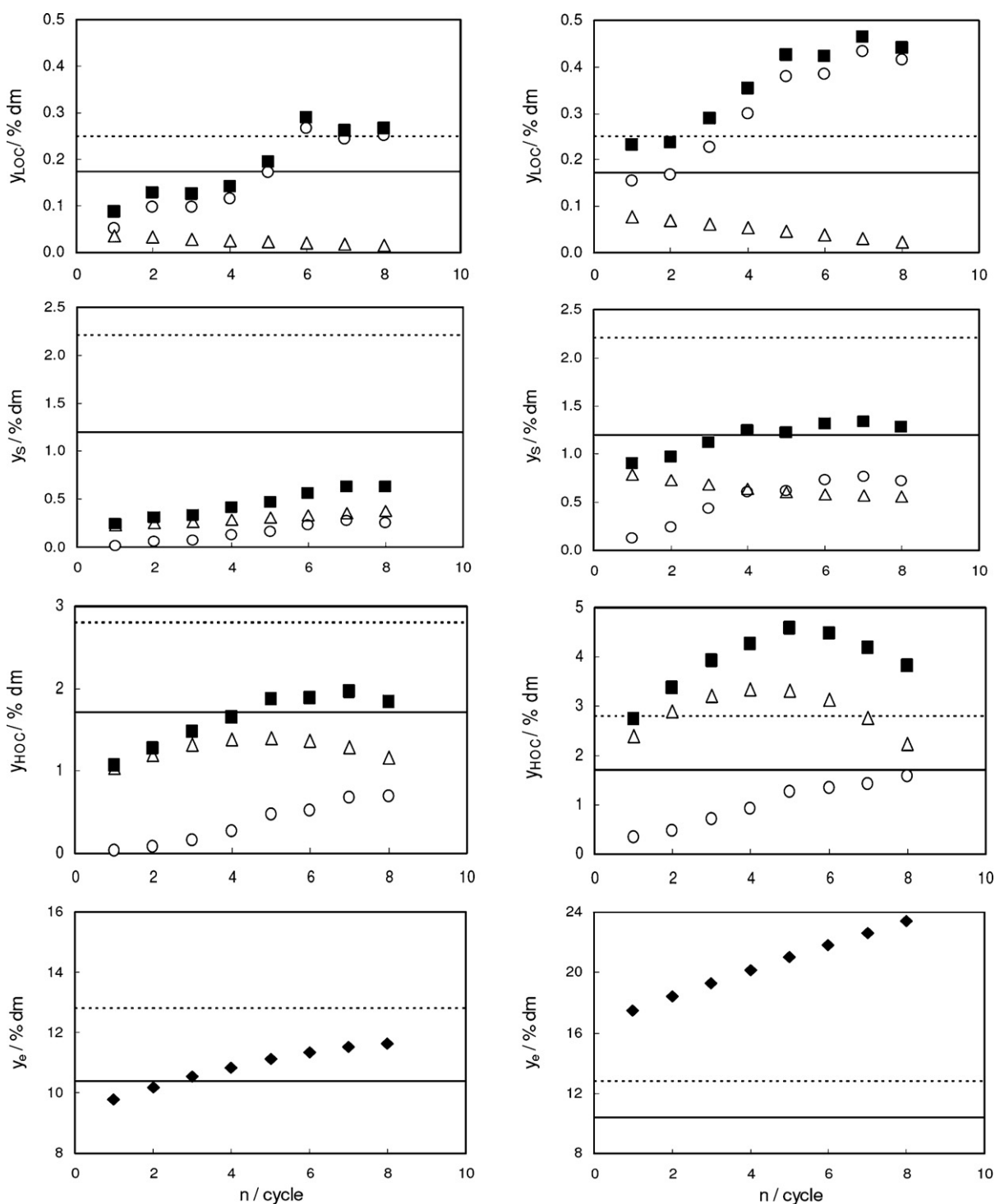


**Fig. 10.** Volatiles and non-volatiles yield as a function of number of DIC cycles during the total heating time of 6 min at 0.28 MPa (left) and 0.6 MPa (right): (○) direct oil yield ($y_{od}$), (△) yield of volatiles in residual extract ($y_{os}$), (■) total yield ($y_T$), (◆) residual extract yield; full line: reference extract (2 h); dashed line: reference extract (8 h) [23].

The residual availability of HOC exhibits a maximum for $n = 5$ (see Fig. 6 and Table 3). The maximum equal to 2.35 is obtained at $p = 0.6$ MPa during 10.3 min. It means that during 2 h chloroform extracted 2.35 times more HOC from DIC-treated flowers than from non-treated flowers. This tendency gives rise to the hypothesis that the majority of HOC are situated in endogenous site, where they

have strong interactions with solid matrix. After a critical damage of endogenous cells by 5 cycles in 10 min, the auto-vaporization of embedded compounds (HOC) became important and their availability decreased. It can be concluded that the amount of vaporized compounds during pressure drop (isolation efficiency) increases with increasing $p$ and $n$.
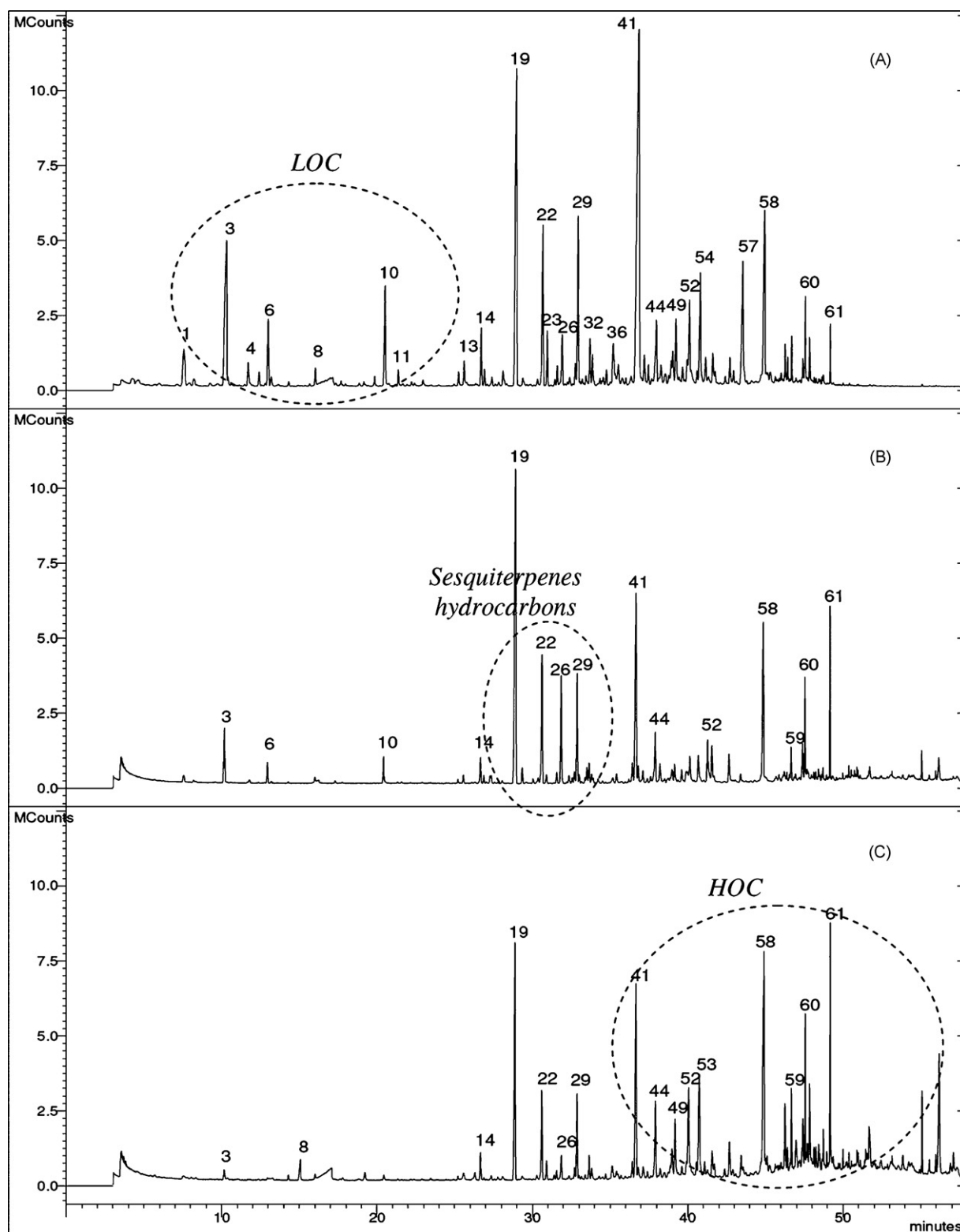


**Fig. 11.** Gas chromatogram of DIC direct oil (A), reference oil (B), and DIC residual oil (C). DIC conditions: $p = 0.6$ MPa, $n = 8$ cycles, $t = 6$ min. See Table 5 for peak number.

The availability of sesquiterpenes hydrocarbons decreases with $n$, while time is not a significant parameter (see Fig. 7). Like in HOC, the effect of $n$ becomes more important with increasing $p$. It can be supposed that the amount of sesquiterpenes hydrocarbons in condensate increases with increasing $n$. It should be mentioned that boiling points of sesquiterpenes hydrocarbons are lower than those of HOC. The availability dependence on $p$ and $n$ gives rise to the conclusion that sesquiterpenes hydrocarbons are located in both exogenous and endogenous sites. Parameters of surface response with corresponding maximal values of $A_S$ are given in Table 3.

The DIC effect on $A_{LOC}$ is similar as on sesquiterpenes hydro-carbons, but the decrease of availability with $n$ is more important than at sesquiterpenes (see Fig. 8). The exogenous state and a higher volatility of LOC result in a greater amount in condensate. At a higher pressure, time is not significant. The parameters of surface response and maximal values of availability are given in Table 3.

**Table 5**
Chemical composition (mass%) of reference oil (non-treated flowers, 2 h extraction), DIC direct and residual oils (0.6 MPa, 8 cycles, 6 min)

| No. | RI | Compounds | Type | DIC direct oil | DIC residual oil | Reference oil |
|---|---|---|---|---|---|---|
| 1 | 961 | Benzaldehyde (179 °C) | OL | 1.29 | 0.00 | 0.38 |
| 2 | 985 | 6-Methyl-5-hepten-2-one | OL | 0.22 | 0.00 | 0.18 |
| 3 | 1032 | Benzyl alcohol (205 °C) | OL | 6.45 | 0.21 | 2.60 |
| 4 | 1074 | cis-Linalool oxide (furanoid form) | OM | 0.53 | 0.02 | 0.06 |
| 5 | 1088 | trans-Linalool oxide (furanoid form) | OM | 0.33 | 0.00 | 0.00 |
| 6 | 1098 | Linalool (198 °C) | OM | 1.56 | 0.04 | 0.75 |
| 7 | 1095 | α-Pinene oxide | OM | 0.21 | 0.04 | 0.05 |
| 8 | 1163 | Benzyl acetate | OL | 0.34 | 0.02 | 0.17 |
| 9 | 1240 | Neral | OM | 0.00 | 0.00 | 0.00 |
| 10 | 1255 | Geraniol (229 °C) | OM | 2.49 | 0.09 | 0.77 |
| 11 | 1270 | Geranial | OM | 0.28 | 0.01 | 0.06 |
| 12 | 1351 | α-Cubebene | S | 0.27 | 0.11 | 0.23 |
| 13 | 1356 | Eugenol | OL | 0.60 | 0.07 | 0.18 |
| 14 | 1376 | α-Copaene | S | 1.16 | 0.53 | 1.18 |
| 15 | 1383 | Geranyl acetate | OM | 0.31 | 0.00 | 0.19 |
| 16 | 1390 | β-Cubebene | S | 0.00 | 0.00 | 0.58 |
| 17 | 1391 | β-Elemene | S | 0.15 | 0.00 | 0.00 |
| 18 | 1401 | Methyl eugenol | OL | 0.45 | 0.00 | 0.10 |
| 19 | 1418 | β-Caryophyllene (260 °C) | S | 10.31 | 8.70 | 18.70 |
| 20 | 1433 | γ-Elemene | S | 0.21 | 0.19 | 0.46 |
| 21 | 1443 | (Z)-β-Farnesene | S | 0.13 | 0.18 | 0.25 |
| 22 | 1454 | α-Humulene | S | 3.90 | 2.88 | 5.86 |
| 23 | 1458 | (E)-β-Farnesene | S | 1.00 | 0.21 | 0.48 |
| 24 | 1461 | Allo-aromadendrene | S | 0.12 | 0.13 | 0.00 |
| 25 | 1477 | γ-Muurolene | S | 0.39 | 0.45 | 0.00 |
| 26 | 1480 | Germacrene D | S | 1.16 | 1.10 | 2.53 |
| 27 | 1490 | cis-β-Guaiene | S | 0.20 | 0.25 | 0.41 |
| 28 | 1499 | α-Muurolene | S | 0.49 | 0.39 | 0.49 |
| 29 | 1500 | trans-β-Guaiene (271 °C) | S | 3.92 | 2.35 | 4.49 |
| 30 | 1509 | β-Bisabolene | S | 0.09 | 0.04 | 0.11 |
| 31 | 1513 | γ-Cadinene | S | 0.20 | 0.27 | 0.23 |
| 32 | 1529 | β-Cadinene (274.5 °C) | S | 0.97 | 1.24 | 0.77 |
| 33 | 1524 | cis-Calamenene + δ-cadinene | S | 0.73 | 0.45 | 0.65 |
| 34 | 1532 | trans-Calamenene | S | 0.18 | 0.18 | 0.15 |
| 35 | 1534 | Cadina-1,4 diene | S | 0.34 | 0.11 | 0.10 |
| 36 | 1534 | (Z)-Nerolidol | OS | 1.35 | 0.42 | 0.49 |
| 37 | 1536 | Dihydro-eugenol acetate | OH | 0.70 | 0.53 | 0.31 |
| 38 | 1549 | Elemol | OS | 0.29 | 0.23 | 0.08 |
| 39 | 1564 | (E)-Nerolidol | OS | 0.16 | 0.00 | 0.04 |
| 40 | 1570 | (Z)-3-Hexenyl benzoate | OH | 0.32 | 0.10 | 0.14 |
| 41 | 1581 | Caryophyllene oxide | OS | 18.84 | 6.64 | 12.03 |
| 42 | 1583 | Globulol | OS | 0.61 | 0.76 | 0.46 |
| 43 | 1596 | Cedrol | OS | 0.36 | 0.28 | 0.22 |
| 44 | 1606 | Humulene epoxide II | OS | 2.05 | 2.23 | 3.04 |
| 45 | 1640 | Epi-α-cadinol | OS | 0.69 | 1.24 | 0.69 |
| 46 | 1642 | Cubenol | OS | 0.27 | 0.58 | 0.30 |
| 47 | 1645 | α-Muurolol | OS | 0.55 | 0.24 | 0.23 |
| 48 | 1649 | β-Eudesmol | OS | 0.85 | 1.23 | 0.48 |
| 49 | 1652 | α-Eudesmol | OS | 1.64 | 1.90 | 0.61 |
| 50 | 1653 | α-Cadinol | OS | 0.46 | 0.98 | 0.45 |
| 51 | 1658 | 7-Epi-α-eudesmol | OS | 0.60 | 0.68 | 0.51 |
| 52 | 1671 | β-Bisabolol | OS | 2.23 | 2.88 | 0.53 |
| 53 | 1672 | Aromadendrene oxide-(1) | OS | 0.48 | 1.47 | 0.75 |
| 54 | 1678 | Aromadendrene oxide-(2) | OS | 3.11 | 2.46 | 2.13 |
| 55 | 1679 | (Z)-α-Santalol | OS | 0.81 | 2.46 | 1.29 |
| 56 | 1688 | 8-Cedren-13-ol | OS | 0.66 | 1.17 | 1.84 |
| 57 | 1722 | (E,E)-Farnesol | OS | 4.13 | 0.82 | 0.45 |
| 58 | 1762 | Benzyl benzoate (323 °C) | OH | 6.31 | 8.86 | 6.74 |
| 59 | 1843 | (E,E)-Farnesyl acetate | OS | 0.68 | 2.05 | 1.25 |
| 60 | 1863 | Benzyl salicylate (300 °C) | OH | 1.52 | 3.50 | 3.05 |
| 61 | 1949 | Geranyl benzoate | OH | 0.57 | 4.98 | 0.47 |

The boiling points of selected compounds are at the atmospheric pressure. OH: other heavy oxygenated compounds; OL: other light oxygenated compounds; OM: oxygenated monoterpenes; OS: oxygenated sesquiterpenes; S: sesquiterpenes hydrocarbons.

The effect of cycle number and pressure on the flower structure is shown in Fig. 9. The micrographs of cross section show that the alveolation increases with increasing cycle number and pressure. In accordance with the trends in RSM analysis results, the alveolated structure facilitates the liberation and vaporization of volatile molecules and simultaneously increases the availability of the residual HOC to solvent extraction from the endogenous site of the glands.

The DIC treatment enhances the availability of HOC in residual flowers. In this case Eq. (9) gives an erroneous estimation of isolation efficiency. For example, the value of $E$ is negative for $A_{HOC} = 2.35$. Hence isolation efficiency can be obtained only by analysis of DIC condensate. The results of this analysis are presented in the next chapter.

### 3.2. Total availability

Our preliminary kinetics study of direct oil isolation demonstrated that the degradation of caryophyllene oxide, benzyl alcohol and geraniol occurs when the heating time of one DIC cycle at 0.6 MPa exceeds 6 min. A significant increase of oil yield occurred in the first 6 min and then the yield was almost constant. This observation was confirmed in the previous chapter, where the time exceeding 6 min had almost no effect. Therefore, in this part, where the both direct oil and residual extract are analyzed a total heating time of 6 min was selected.

The yield of volatiles in direct and residual oils and the yield of residual extract are shown for two pressures in Fig. 10 as a function of cycle number. There are also reference values obtained by chloroform extraction during 2 and 8 h [23]. The later values are the limiting values which can be obtained by chloroform extraction. The yield of direct oil compounds and extract increases significantly with $p$ and $n$. At 0.6 MPa, the yields of extract and direct LOC ($n > 3$) are superior to their yield in 8 h reference. As found in the previous chapter, the residual LOC is present in a small quantity which decreases with $n$. The yield of direct HOC attains the 2 h reference value for 0.6 MPa and 8 cycles. The rest of HOC which is located in endogenous sites can be extracted only by solvent. The maximum of residual HOC on the cycle dependence ($n = 5$) is due to the increasing amount of evaporated compounds (direct oil). The total yields of HOC and LOC are higher than the 8 h reference yield.

Contrary to HOC and LOC, sesquiterpenes hydrocarbons fulfil the hypothesis $y_{jd} + y_{js}$ (2 h) $= y_{jf}$ (2 h) for 0.6 MPa. In other words, isolation efficiency can be calculated from their content in residual flowers using Eq. (9). The high quantity of S in the 8 h reference extract is probably due to a long contact of material with hot solvent which leads to degradation of other compounds into S. A short heating period during the DIC treatment reduces these transformations.

For 0.6 MPa, the total yields of LOC and HOC are higher than the limiting reference value of chloroform extraction (8 h). The total yield of sesquiterpenes hydrocarbons is equal to the reference value of 2 h extraction. In conclusion, the total availability ($A_T$ defined by Eq. (8)) of LOC, S and HOC ($p = 0.6$ MPa, 8 cycles, 6 min) are 2.5, 1.0 and 2.2, respectively.

The oxygenated compounds (LOC and HOC) are highly odoriferous, while the sesquiterpenes hydrocarbons contribute only little to fragrance and therefore are less valuable. The quality of cananga oil increases with the content of oxygenated compounds which is the case of DIC treatment. The amount and composition of reference oil and DIC direct and residual oils are compared in Table 4. The corresponding chromatograms are shown in Fig. 11. The direct oil contains much more LOC than the other oils. The residual oil contains less oxygenated monoterpenes, other light oxygenated compounds and sesquiterpenes hydrocarbons than the reference oil. A significant quantity of non-identified other heavy oxygenated compounds with elution time between 45 and 50 min was found in the residual oil, in contrast to their negligible quantity in the reference oil. The peak identification and detailed chemical composition can be found in Table 5.

A direct oil yield of 2.77% dm was isolated using DIC at 0.6 MPa and 8 cycles in 6 min. This yield is higher than a yield of 2.54% dm isolated in 16 h using steam distillation (SD) [23]. The DIC direct oil contains more LOC and HOC than SD oil (15%; 57.5% versus 7.4%; 54.3%). A similar result, i.e. an increase of oxygenated compounds, was obtained by Lucchesi et al. [14] using solvent-free microwave extraction. On the other hand, the sesquiterpenes hydrocarbons are present in a higher quantity in SD oil (38.4% versus 25.9%). The DIC process yields more valuable cananga oil than SD.

In our experiments, a mass of 40 g of dry cananga flowers was treated in the 6 L autoclave. A layer of 70 mm of flowers was placed in a perforated steel container with a diameter of 110 mm. In scaling-up of DIC process, steam penetration and heat transfer in the flowers are limiting factors. In scaling-down, the quantity of condensed steam will increase with increasing ratio surface/volume. Hence a sufficient heating of the autoclave must be assured by a double jacket.

## 4. Conclusions

The DIC isolation process is based on auto-vaporization of super-heated volatile compounds from modified structure resulted from multi-cycle pressure drop and not on molecular diffusion as the classical separation methods. Thermal degradation risk is avoided by using a short heating time (<1 min) in each DIC cycle.

The availability of HOC in residual flowers after DIC treatment exhibits a maximum of 2.35 for 5 cycles at 0.6 MPa. The maximum on the cycle dependence is due to the increasing amount of compounds which leave the flowers with vapor. The residual availability of sesquiterpenes hydrocarbons and LOC, which are more volatile than HOC, increases with increasing pressure and decreases with the cycle number. The smaller boiling point the more compounds leave the flowers with vapor during the pressure drop.

Oxygenated monoterpenes, other light oxygenated compounds, sesquiterpenes hydrocarbons and a part of oxygenated sesquiterpenes and other heavy oxygenated compounds in exogenous sites and a little quantity in endogenous ones are isolated as direct oil by instantaneous auto-vaporization. The DIC treatment increases simultaneously the availability of non-volatiles compounds and oxygenated sesquiterpenes and other heavy oxygenated compounds to the solvent extraction from the endogenous sites of residual flowers. The porosity of flowers increases significantly with steam pressure and cycle number.

In comparison with steam distillation, the DIC process (0.6 MPa, 8 cycles) was more rapid (6 min versus 16 h) and gave higher direct oil yield (2.77% dm versus 2.54% dm) which contained more oxygenated fractions (72.5% versus 61.7%). The sum of LOC and HOC in the DIC direct and residual oils was greater than their content in the reference oil obtained by chloroform extraction from non-treated flowers.

The DIC process is very efficient for essential oil isolation and can be also used as a pre-treatment for improvement of the extraction of non-volatiles compounds.

### Notation

$A_j$        availability of compound $j$ in residual flowers (ratio of peak areas of compound $j$ in residual and reference extracts, $A_j = S_{js}/S_{jf}$)

$A_T$     total availability (the sum of the volatiles mass in DIC condensate and residual flowers/volatiles in 2 h reference extract)

$E$     DIC isolation efficiency, $E_j = y_{jd}/y_{jf}$

$m$     mass

$n$     number of DIC cycles

$N$     number of independent variables

$p$     pressure

$S$     peak area

$t$     heating time

$T$     temperature

$x_j$     mass fraction of compound $j$ in oil (peak area of $j$ divided by the sum of peaks of all volatiles, $x_j = S_j / \sum_l S_l$

$X$     independent variable

$y_e$     extract yield (mass of extract/dry matter of flowers)

$y_j$     yield of compound $j$ ($y_j = x_j y_{oil}$)

$y_o$     oil yield (mass of volatiles in extract/dry matter of flowers)

*Greek letters*

$\alpha$     maximal axial distance of parameters from the central point

$\beta$     regression coefficient

$\eta$     dependent variable

*Subscripts*

A     autoclave

d     DIC direct oil, isolated from DIC condensate

dm     dry matter of flowers

e     extract (total mass of the extract comprising volatile oil and non-volatile molecules)

f     reference extract of non-treated flowers

j     compound $j$

o     oil (all volatiles compounds in extract)

P     product

T     total volatiles compounds in flowers

V     reservoir

s     residual extract of DIC-treated flowers

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