Bionanomaterials: Synthesis, Physico-Chemical and Multivariate Analyses of the Dicotyledonatae and Pinatae Essential Oil/ β - cyclodextrin Nanoparticles

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The paper presents a multivariate analysis (PCA-principal component analysis) of the essential oils from Dicotyledonatae and Pinatae classes encapsulated in β-cyclodextrin. The essential oil/β--cyclodextrin complexes were obtained by solution method. Uncomplexed volatile oils and complexes with β--cyclodextrin were analyzed by gas chromatography-mass spectrometry (GC-MS) and thermogravimetry (TG), respectively. In order to evaluate the composition of the encapsulated oil, this was extracted from the complex and analyzed by GC-MS. For the GC data of the uncomplexed essential oils and the complexed ones, a powerful multivariate statistical procedure (PCA) was applied. Samples were very good classified in botanical classes or in uncomplexed and complexed ones using the GC data of the monoterpene compounds.

Keywords: β--cyclodextrin, essential oils, encapsulation, thermogravimetry, gas chromatography-mass spectrometry, multivariate analysis, PCA

In the last years, the protecting matrices of the bioactive molecules were widely studied. Some of these matrices, with good protective and controlled release properties are cyclodextrins (CDs), which are natural cyclic oligosaccharides, containing six (αCD), seven (βCD), eight (γCD) or more glucopyranose moieties. These compounds have structures like truncated cones, with hydrophobic inner cavities, which can interact with hydrophobic organic molecules and form a more stable, protecting (to air, temperature, light), and controlled releasing supramolecular system [1-3].

Some studies were performed especially in the pharmaceutical field (the complexation of cyclodextrin with different drugs) [4-7], in the food industry (to increase the stability of the aroma, to mask disagreeable taste, and to enhance the quality of foods) [1, 8, 9]; other applications of cyclodextrins are in cosmetic, agricultural, and tobacco industries.

In our previous work we have been obtained and characterized complexes of odorant and flavoring systems or unsaturated fatty acids [10-15] with cyclodextrins. The study on the flavoring systems/cyclodextrin complexes was extended to the nanoencapsulation of the essential oils from the Rutaceae and Myrtaceae family plants (Dicotyledonatae class) and Cupressaceae family plants (Pinatae class) in β-cyclodextrin in order to evaluate the concentration and the composition of the encapsulated essential oils and to identify the main components responsible for the multivariate statistical classification [16[.

Materials and method

Essential oils used for the nanoencapsulation were obtained from SC Natex SA Cluj Napoca, România (bergamot, lemon, orange, clove, eucalyptus, and juniper-plant essential oils) and from SC Fares SA Orã°tie, România (juniper-leaf and fruit essential oils). β-Cyclodextrin (βCD, >99%, reagent grade) were purchased from Merck&Co., Inc, New Jersey. Alkane Standard solution C_8-C_{20}, used for Kovats index determination, was obtained from Fluka ChemieAG.

Complexation method. 0.5 mmole of β-cyclodextrin (table 1) were dissolved in 8 mL distilled water at 50±1°C in a thermocontrolled minireactor, equipped with a reflux condenser, and then 5 mL ethanolic solution of the essential oil (the weight corresponding to essential oil main compound : βCD molar ratio of 1 : 1, table 1) were slowly added under continuous stirring. The solution was then stirred another 15 min and slowly cooled at 20°C in about 4 h. The crystallization was perfected in refrigerator at 4°C for 24 h. The complex was filtered, washed with ethanol and dryied in exicator.

Table 1
QUANTITIES OF ESSENTIAL OILS FROM RUTACEAE, MYRTACEAE, AND CUPRESSACEAE FAMILY PLANTS, β CD USED FOR ENCAPSULATION, AND YIELDS FOR THE ENCAPSULATION PROCESS.

No	Complex	m (ess.oil) (mg)	m (βCD) (mg)	m (complex) (mg)	Yield (%)
1	bergamot ess.oil/βCD	68.3	671.0	563.8	76.3
2	lemon ess.oil/βCD	70.7	671.0	404.7	54.6
3	orange ess.oil/βCD	72.4	671.5	557.6	75.0
4	clove ess.oil/βCD	82.7	671.8	469.1	62.2
5	eucalyptus ess.oil/βCD	80.3	671.1	632.1	84.1
6	juniper-plant ess.oil/βCD	70.9	671.4	589.6	79.4
7	juniper-leaf ess.oil/βCD	87.2	670.8	549.9	72.6
8	juniper-fruit ess.oil/βCD	71.6	671.0	579.0	78.0

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Essential oil extraction from the complex. 100 mg Essential oil/βCD complex was dissolved in 4 mL distilled water in a thermocontrolled minireactor, equipped with a reflux condenser and a magnetic stirrer; 2 mL hexane was then added and the emulsion was vigorously stirred for 20 minutes at 69°C. After cooling, the organic layer was separated and the aqueous layer was extracted for another three times with 3 . 2 mL hexane in the same manner. All organic layers were dryied over anhydrous $CaCl_2$ and analyzed by GC-MS.

TG analysis.

The thermogravimetric analysis for the essential oil/βCD complexes was performed on a Netzsch TG-209 apparatus, using a temperature program of 20-200°C, with a heating rate of 4°C/min, and from 200 to 900°C with a heating rate of 10°C/min. All analyses were conducted under nitrogen atmosphere.

GC-MS analysis.

For the analysis of the raw and recovered essential oils a Hewlett Packard HP 6890 Series gas chromatograph coupled with a Hewlett Packard 5973 mass spectroscopy detector (GC-MS) system was used. A HP-5 MS capillary column (30 m length, 0.25 mm i.d., 0.25 μ m film thickness) was used for the GC system. The temperature program was set up from 50°C to 250°C with 4°C/min, both the injector and detector temperatures were 280°C and He was used as carrier gas. The injection volume was 2 μL. Ionization energy EI of 70eV was used for mass spectroscopy detector, with a source temperature of 150°C, scan range 50-300 amu, scan rate $1s^{-1}$. Compounds separated by GC were identified by matching the experimental mass spectra with those from the NIST/EPA/NIH Mass Spectral Library 2.0 and by comparing the Kovats indices of the separated compounds with those of the standard compounds (from our database of odorant and flavoring compounds) for the same GC column (HP-5). The quantification was performed by GC area method, using a calibration factor of 1.0 (dodecane was used as external standard).

Principal Component Analysis (PCA)

The multivariate analysis (PCA) of the GC data for raw and recovered essential oils was performed in order to classify the essential oils used for nanoencapsulation and to identify the important compounds for the classification. We have used a modified *in house* program with centered data and cross-validation method for the validation.

Results and discussions

For the complexation process the best yields were obtained in the case of eucalyptus oil, bergamot oil, orange oil and all juniper oils (84%, 76%, 75%, and 73-79%, respectively). In the case of lemon and clove oils lower yields were obtained (55% and 62%, respectively). The thermogravimetric analysis indicates that the concentration of encapsulated compounds is in the range of 7.5-11.5% for Dicotyledonatae class (fig. 1) and 7.1-8.1% for the Pinatae class (fig. 2).

In all cases from the Rutaceae family essential oils limonene was the main compound (55% for the lemon oil and 88% for the orange oil). For the bergamot oil the concentration of limonene was 17%, the most concentrated compound being linalool (22%). A very large number of compounds were separated by GC (over than 80, figs. 3-5), but only the most concentrated ones are presented in Table 2. All main compounds were encapsulated in aproximately the same relative concentrations (% w/w, concentration determined in the hexane extract from the complex, using the peak area method and a calibration factor of 1.0) in the case of bergamot oil, only α-pinene being more concentrated in the complex (table 2). In the case of oils with high concentration of limonene (lemon and orange oils), this compound was encapsulated in higher relative concentration.

The most concentrated compounds from the Myrtaceae family essential oils were eugenol (78% in the clove essential oil) and eucalyptol (86% in the eucalyptus essential oil) (figs. 6, 7 and Table 2), both compounds being encapsulated in approximately the same relative concentrations comparing with those from the raw essential oils (table 2).

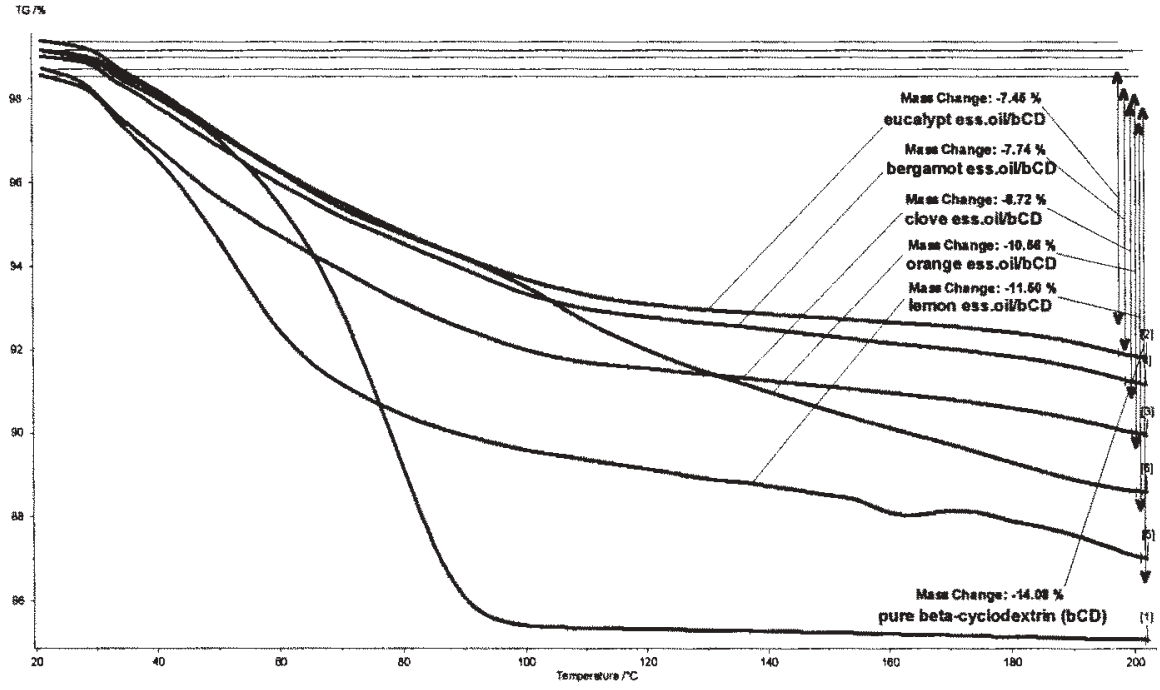

Fig. 1. TG analysis of the essential oils from the Dicotyledonatae class/βCD complexes

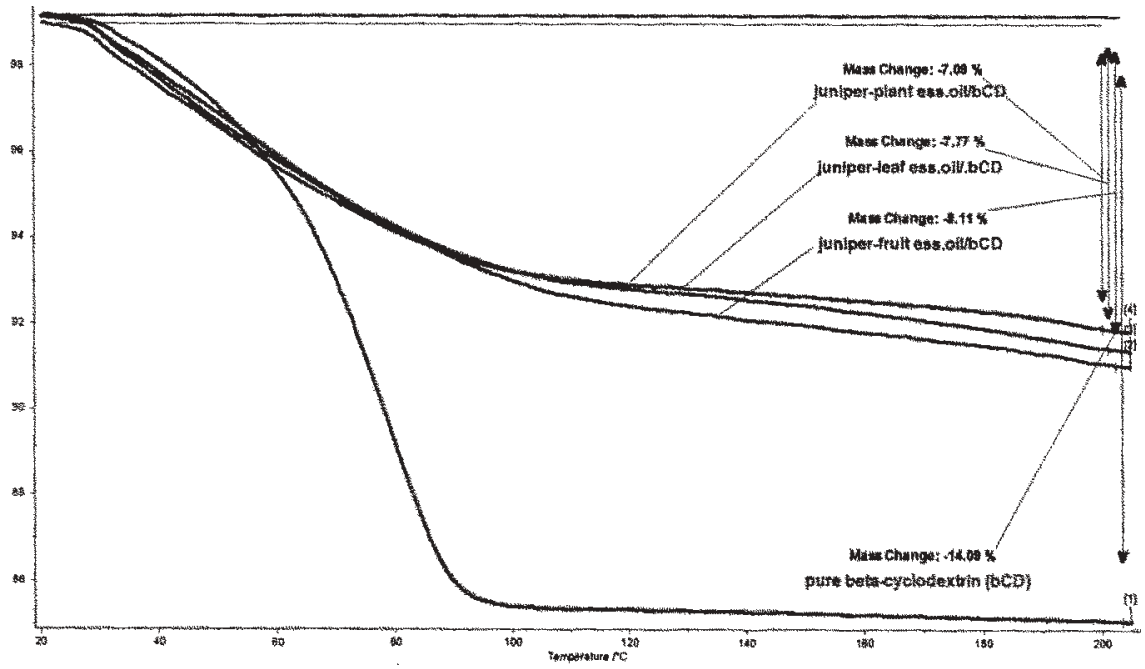

Fig. 2. TG analysis of the essential oils from the Pinatae class/βCD complexes

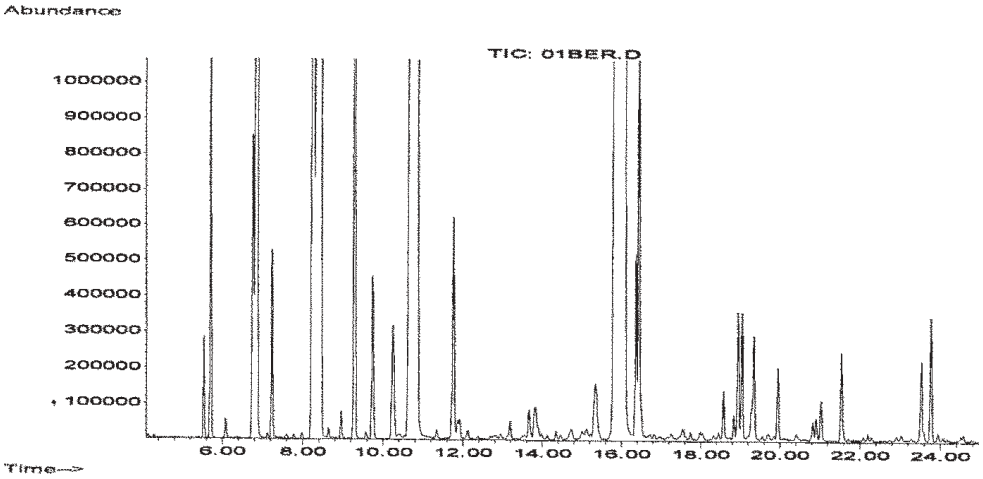

Fig. 3. Gas chromatogram from the GC-MS analysis of the bergamot essential oil

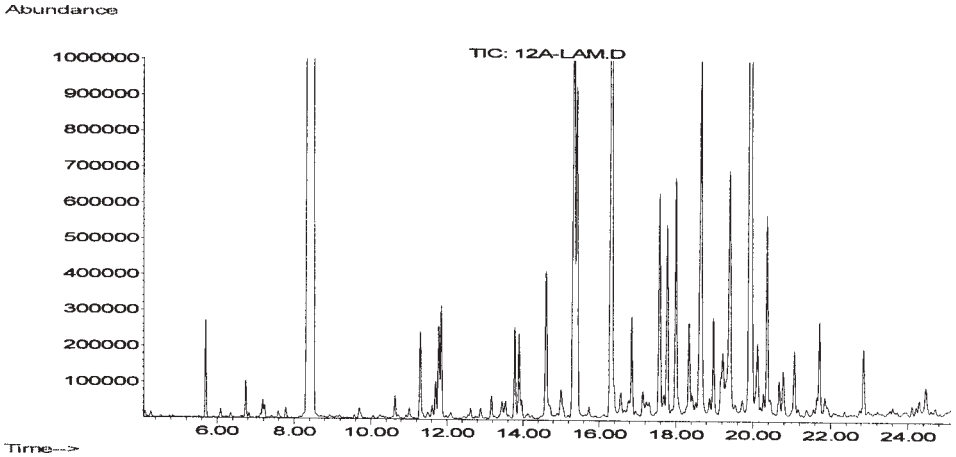

Fig. 4. Gas chromatogram from the GC-MS analysis of the lemon essential oil

All juniper oils (Pinatae class) have α-pinene as main compound, a very large number of compounds being separated by GC (over than 130, fig. 8). The GC profile of these oils were approximately the same, only in the case of juniper-leaf essential oil β-phellandrene being more concentrated (table 3). In the case of juniper-fruit essential oil relatively high concentrations of humulene, β-cubebene, and γ-elemene were observed (table 3). α-Pinene was encapsulated in double relative concentrations in complexes in all cases. Terpenic alcohols and sesquiterpenes were encapsulated in lower concentrations, probably due to the low hydrophobic interaction with the inner βCD cavity or to the higher size of the molecules (in the case of sesquiterpenes).

The multivariate analysis (PCA-principal component analysis) of the GC data (concentration of the compounds), both for the raw and recovered essential oils from the Rutaceae, Myrtaceae, and Cupressaceae family plants (Dicotyledonatae and Pinatae classes), indicate a good classification of these botanical families. The Pinatae class

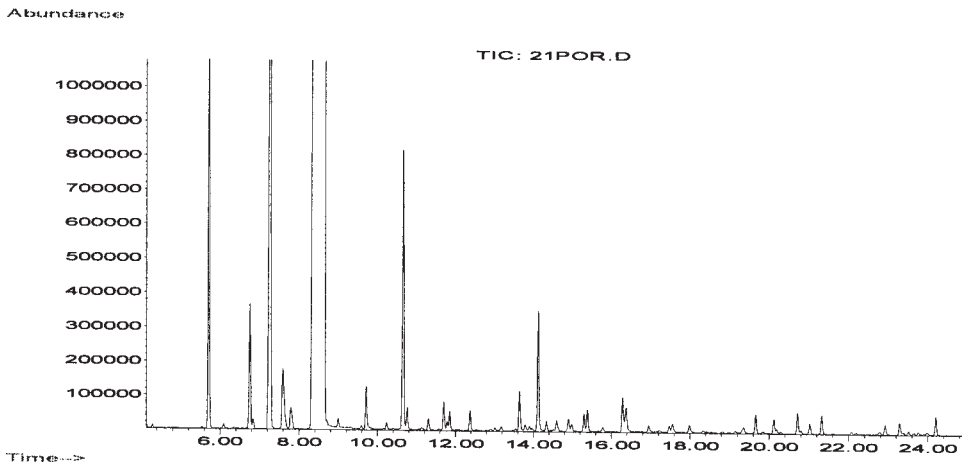

Fig. 5. Gas chromatogram from the GC-MS analysis of the orange essential oil

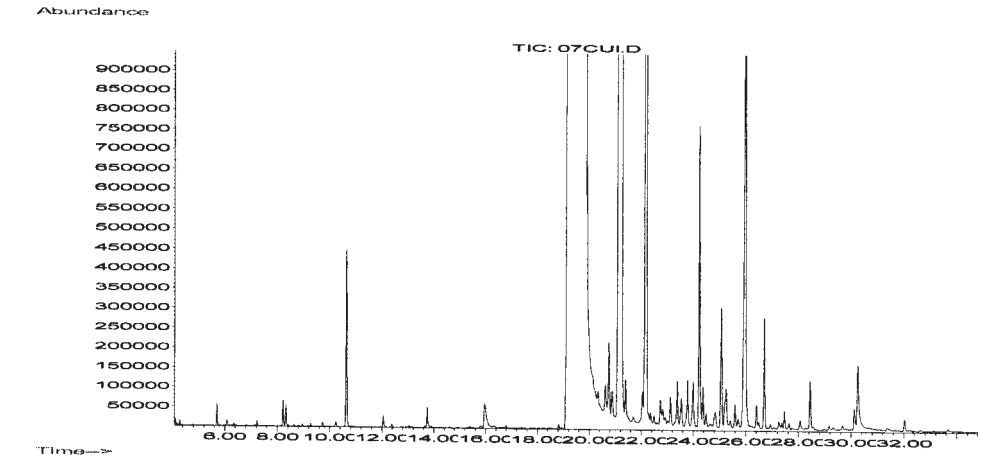

Fig. 6. Gas chromatogram from the GC-MS analysis of the clove essential oil

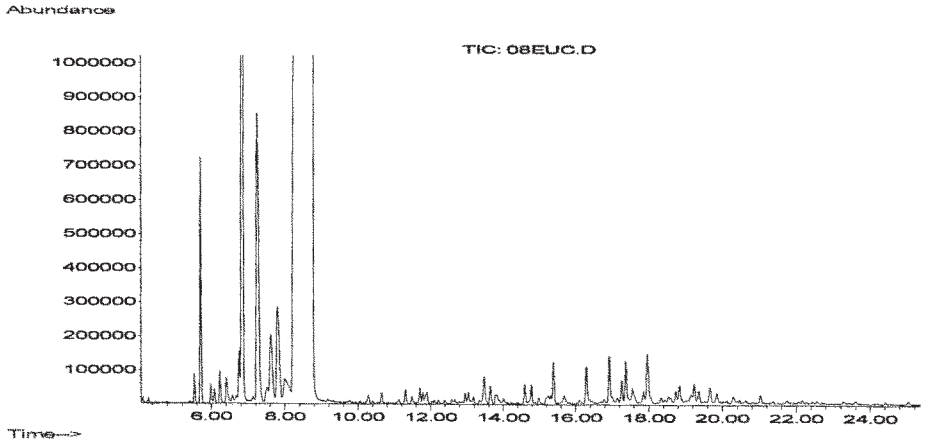

Fig. 7. Gas chromatogram from the GC-MS analysis of the eucalyptus essential oil

Table 2

CONCENTRATION (%, w/w) OF THE MAIN COMPOUNDS IDENTIFIED BY MS OR KI (VALUE IN PARENTHESIS) IN RAW ESSENTIAL OILS AND IN THE RECOVERED ONES (FOR DICOTYLEDONATAE CLASS)

Bergamot	Lemon	Orange	Clove	Eucalyptus
β-pinene	*α*-pinene	*α*-pinene	eugenol	*β*-pinene
7.2/3.5 (980)	0.4/0.6 (936)	1.7/0.6 (936)	78/64 (1381)	4.2/1.8 (979)
p-cymene	limonene	myrcene	*β*-caryophyllene	*p*-cymene
3.1/3.3 (1027)	55/87.6 (1040)	4/1.7 (993)	13.5/28 (1427)	1.8/9.8
limonene	neral	limonene	humulene	eucalyptol
17/14.8 (1034)	4.2/2.2 (1244)	88/96 (1034)	3.7/5.5 (1458)	86/86 (1038)
γ-terpinene	carvone	linalool	caryophyllene-	other compds.
2.2/2.9 (1061)	2.2/1.8 (1246)	1.3/0.4 (1103)	oxid 1.3/0.8	8/2.4
linalool	geranial	other compds.	other compds.	
22/19.5 (1109)	5.8/2.6 (1274)	5/1.3	3.5/1.7	
other compds.	other compds.			
48.5/55.3	32.4/5.2			

(P) was very good classified in the centre of the PC2 *vs* PC1 loadings plot (fig. 9) and the Dicotyledonatae class (D) was grouped in two region: the left large distributed region and the right zone along to the PC1 component. These two principal components explain 97% from the variance of the data (93% PC1 and 4% PC2). The most

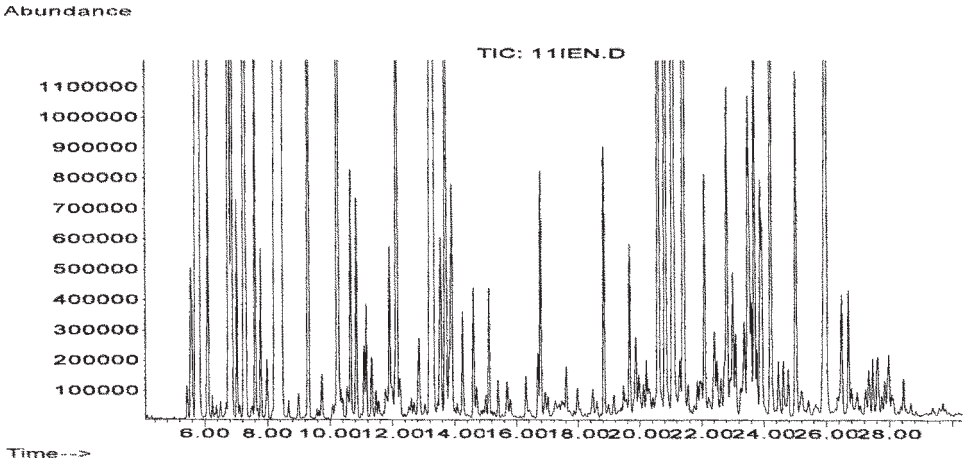

Fig. 8. Gas chromatogram from the GC-MS analysis of the juniper-plant essential oil

Juniper-plant	Juniper-leaf	Juniper-fruit
α-pinene	α-pinene	α-pinene
16/37 (941)	14.4/28 (940)	15.3/39.8 (942)
β-phellandrene	β-phellandrene	β-phellandrene
3.6/2.8 (977)	14.8/23 (979)	6.8/7.6 (979)
β-pinene	β-pinene	β-pinene
2.8/3 (980)	1.5/1.6 (981)	2.1/2.4 (981)
myrcene	myrcene	myrcene
4/3.7 (995)	3.5/3.1 (994)	6.4/7.4 (997)
p-cymene	*p*-cymene	*p*-cymene
7.4/7.3 (1029)	4.3/3.8 (1028)	1.9/1.5 (1027)
limonene	limonene	limonene
9.9/13.3 (1034)	7.3/7.1 (1033)	4.8/4.5 (1033)
γ-terpinene	γ-terpinene	γ-terpinene
2.2/1.8 (1061)	7.3/6.8 (1063)	3.5/2.6 (1062)
terpinolene	terpinolene	terpinolene
3/2.2 (1091)	4.4/3.5 (1091)	2.7/1.8 (1091)
cis-verbenol	*cis*-verbenol	terpinen-4-ol
1.5/0.6 (1148)	0.1/0.1 (1147)	4.1/2 (1181)
terpinen-4-ol	terpinen-4-ol	α-terpineol
7.5/6.2 (1183)	7.3/3.8 (1182)	0.5/0.1 (1193)
α-terpineol	α-terpineol	β-caryophyllene
1.6/0.6 (1195)	0.5/0.1 (1193)	3.3/2.8 (1422)
α-cedrene	α-cedrene	humulene
2.5/1.9 (1414)	0.02/- (1414)	2.7/2.3 (1456)
β-caryophyllene	β-caryophyllene	β-cubebene
4.7/3.7 (1422)	1.2/0.7 (1420)	4.5/3.8 (1485)
caryophyllene-	caryophyllene-	γ-elemene
oxid 3.2/1.1	oxid 0.2/-	4.1/2.4 (1560)
other compds.	other compds.	other compds.
30.1/14.8	33.2/18.4	37.3/19

Table 3
CONCENTRATION (%, W/W) OF THE MAIN COMPOUNDS IDENTIFIED BY MS OR KI (VALUE IN PARENTHESIS) IN RAW ESSENTIAL OILS AND IN THE RECOVERED ONES (FOR PINATAE CLASS)

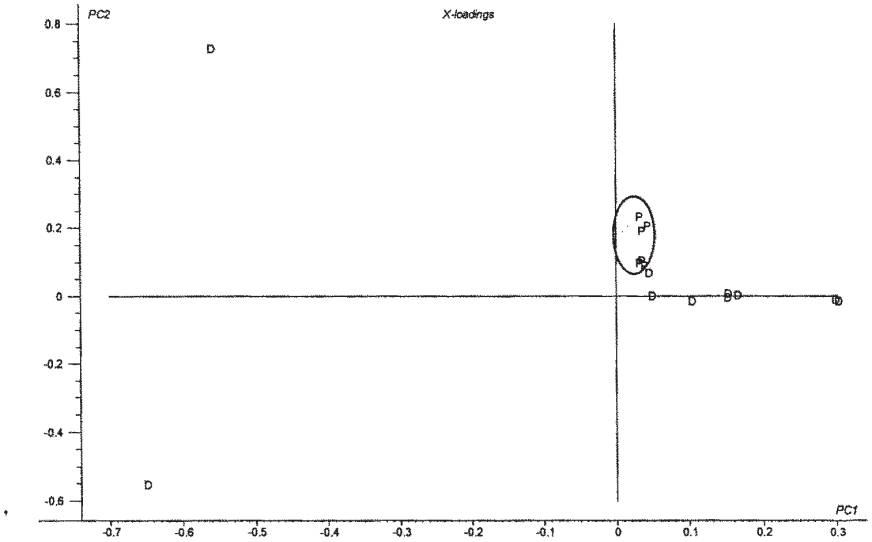

Fig. 9. PC2 *vs* PC1 loadings plot for the GC data of the essential oils from the Dicotyledonatae (D) and Pinatae (P) botanical classes.

important compounds for the classification of the botanical classes are limonene and eucalyptol for the first principal component (PC1), and α-pinene, geranial and neral for PC2. An attempt to classify the raw essential oils (VO) and the recovered ones from the complexes (C) provides good results, these two types of samples being grouped (but not

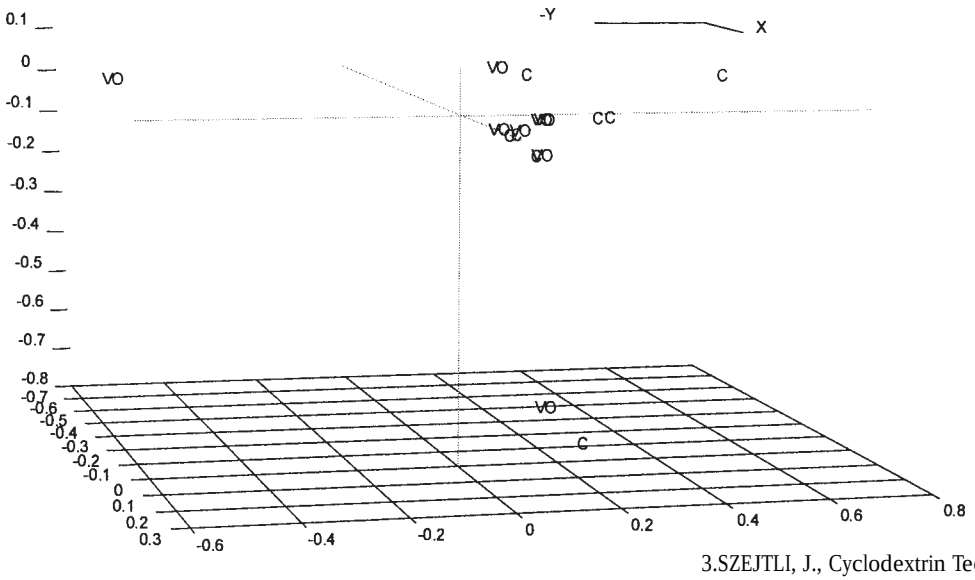

Fig. 10. PC3 *vs* PC1,PC2 loadings plot for the GC data of the raw essential oils (VO) and recovered ones (C) from the Dicotyledonatae and Pinatae botanical classes

very clear) in the left region of the PC3 *vs* PC1,PC2 loadings plot in the case of raw essential oils (VO) and in the right region for the recovered essential oils (C) (fig. 10). This grouping can be clearly observed in the case of Cupressaceae family essential oils (juniper) along the PC2 (Y in the fig. 10) axis.

Conclusions

The nanoencapsulation of the essential oils from the Dicotyledonatae (bergamot, lemon, orange, clove, and eucalyptus) and Pinatae (juniper-plant, leaf, and fruit) was performed with good yields (55-84%), the concentration of the encapsulated sample being in the range of 7.1-11.5%.

The main compounds in the essential oils from Rutaceae family plants were monoterpenes (especially limonene), but a relatively high concentration of citral was determined in the case of lemon essential oil (4.2% neral and 5.8% geranial). The oxygenated compounds were the main components in the essential oils from the Myrtaceae family plants (eugenol and eucalyptol). A large number of compounds (especially mono- and sesquiterpenes) were observed in the case of essential oils from the Cupressaceae family plants, the main compounds being α-pinene, limonene, and β-phellandrene. All concentrated monoterpenic hydrocarbons were encapsulated in higher relative concentrations, especially α-pinene, that was encapsulated in approximately double relative concentration comparatively with the corresponding concentration in the juniper raw essential oils. Generally, limonene was encapsulated in relative higher concentration in the case of Rutaceae essential oils, but the oxygenated compounds were encapsulated in the same relative concentrations as in the raw essential oils (from Myrtaceae family plants).

From the multivariate analysis of the GC data, the botanical classes were clearly classified by limonene, eucalyptol, α-pinene, geranial and neral, but the attempt of grouping the raw and recovered essential oils provides only poor classifications. Very good results were obtained in the case of Cupressaceae family, which are clearly grouped in two classes (raw and recovered essential oils) by α-pinene.

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