Essential oils of *Rosmarinus officinalis* L., effect of harvesting dates, growing media and fertilizers

M.G. MIGUEL, C. GUERRERO, H. RODRIGUES, J. BRITO
Faculdade de Engenharia de Recursos Naturais,
Universidade do Algarve, Campus de Gambelas, 8005-139 Faro,
PORTUGAL

F. DUARTE, F. VENÂNCIO, R. TAVARES
INETI, IBQTA, Departamento de Tecnologia das Indústrias Químicas,
Estrada do Paço do Lumiar, 1699 Lisboa Codex,
PORTUGAL

Abstract: - The effects of the harvesting date, growing media and fertilizers on the yield and chemical composition of the essential oils of *Rosmarinus officinalis* L. maintained in pots were evaluated. The highest oil yield was always obtained in June, being superior or equal to 1 %. Myrcene was the major component present in the essential oils with concentrations always exceeding 20 %, independent on the factors studied. 1,8-Cineole (8.0-12.2 %), α-pinene (8.8-16.5 %) and, in some instances, camphor (4.4-14.1 %) could be also detected in considerable amounts, however never exceeding the myrcene percentages. The amounts of camphor (4.4-8.5 %) were always inferior to the 1,8-cineole levels (8.8-11.8 %) in the local sandy soil regardless the fertilization used. Generally, the lowest levels of myrcene and verbenone were observed during January and April while the highest amounts of 1,8-cineole was observed in April or June. The yield and the chemical composition of the rosemary oils seemed to be more sensitive to the temperature, photoperiod and collection period than to the growing media material or to the fertilization used.

Key words: - Forest externalities, essential oil, medicinal plant, biodiversity, conservation.

1 Introduction

The Mediterranean countries possess adequate climatic and soil conditions for the development of a particular flora, namely aromatic plants. *Rosmarinus officinalis* L., an aromatic plant belonging to the *Labiatae*, is endogenous to Europe, Asia and Africa, mainly in areas surrounding the Mediterranean Sea [1]. However, this aromatic plant can also be found in other countries such as Argentina, Brazil, Uruguay, among others [2,3].

Several countries cultivate rosemary as its essential oils can be used in food, perfume, cosmetic and pharmaceutical industries [2,3].

Several studies regarding the chemical composition of the essential oils of *R. officinalis* L. from different geographic origins have been performed and demonstrated some chemical variability [1,4-8]. The Portuguese rosemary oil is characterized, for example, by relative high amounts of myrcene [1].

In this work, *R. officinalis* L. was maintained in pots in different growing media material and submitted to different types of fertilization. The plants were then harvested at different periods and the oil composition was compared.

2 Material and methods

2.1 Plant material

Terminal cuttings from mature field-grown plants of *Rosmarinus officinalis* L., located at Nave do Barão (Algarve, Portugal) were collected, in December 1996. Each 5-cm long cutting was placed into an alveolar 0.05 L nursery container filled with 75% non-fertilized peat (De Baat H2 source) with 25% coarse perlite, pH from 5.8 to 6.5. The cuttings were maintained in a greenhouse under a temperature of 10-15ºC and 50% humidity and were sprayed with water every week (1 L/nursery container). After 90 days without fertilization, three rooted cuttings were transplanted to 2.5 L pots and placed under outside conditions. Means daily air temperature and mean monthly insolation hours during the experimental period (April 1997 to October 1998) are presented in Figures 1 and 2, respectively. The plants were

distributed in three groups of eighteen pots each according to the type of the growing media material used: a local sandy soil (**A**), a fertilized peat (De Baat N2, 20% black peat and 80% white (blonde) peat, pH from 4.8 to 5.2, fertilization N:P:K 1.0 $kg.m^{-3}$, M. De Baat bv) (**B**) and a non-fertilized peat (Shamrock, 100% white peat, pH from 4.8 to 5.0, Bord na Móna) (**C**). Each growing media material group was divided in two plots and fertirrigated (200/ml pot) fortnightly with two different fertilization solutions: $5g.L^{-1}$ of Hakaphos 15:05:30 with 2% Mg (**F1**) and Hakaphos 13:40:13 with 0.4% Mg (**F2**). The assay had six treatments with nine repetitions (growing media material x fertilization solution).

During the harvesting months (October 1997, January, April and June 1998), the plant material was obtained by cutting the aerial parts of the undershrubs from which the essential oils were obtained as described below. The assay was carried out at Campus de Gambelas, Algarve, at see level, longitude 7º58'31'' W and latitude 37º02'35'' N.

2.2 Isolation procedure

The oils were isolated from fresh material by hydrodistillation, for four hours, using a Clevenger-type apparatus, according to the procedure described in the European Pharmacopoeia [9].

Some data are not shown because the plant material was not enough for the oil isolation and chemical analysis.

2.3 Analysis of the essential oils

2.3.1 Gas chromatography

The gas chromatographic analyses were performed using a Hewlett Packard 5890 Series II gas chromatograph equipped with a FID, a data handling system and a OV-101 fused silica column (30 m x 0.25 mm; film thickness 0.25 μm). Oven temperature was held at 70ºC for 5 min and then programmed to 220ºC at $2ºC.min^{-1}$. Detector and injector temperatures were set at 260ºC and 250ºC, respectively. The carrier gas was helium and the working flow was $1\ mL.min^{-1}$. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from each oil without using correction factors.

2.3.2 Gas chromatograph-mass spectrometry

The GC/MS analyses were performed using a Perkin Elmer 8320 gas chromatograph, equipped with a OV-101 fused silica column (30 m x 0.25 mm; film thickness 0.25 μm) and interfaced with a Finnigan MAT 800 Ion Trap Detector (ITD; software 4.1). Oven temperature was held at 70ºC and programmed to 180ºC at $3ºC.min^{-1}$. Transfer line temperature, 250ºC; ion trap temperature, 220ºC; carrier gas helium adjusted to a linear velocity of $30\ cm.s^{-1}$; splitting ratio, 1:100; ionization energy, 70 eV; ionization current, 60 μA; scan range, 30-400 amu; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C_9-C_{21} *n*-alkanes, and mass spectra with corresponding data of components from reference oils.

The components considered in the present work are those whose concentrations reached 1 % at least once.

3 Results

The highest oil yields were observed in June with percentages greater or equal to 1 %, independent on the growing media or fertilization type used in the experiment (Tables 1 and 2). The major components of the oils were myrcene (21.6 - 30.0 %), α-pinene (8.8-16.5 %) and 1,8-cineole (8.0-12.2 %). In some treatments, the percentages of camphor were also in relatively high amounts, being similar or even exceeding the 1,8-cineole content. Such data were more evident in some of the essential oils obtained from the plants maintained either in the non-fertilized or in fertilized peat and in both fertirrigation types. Only the plants maintained in sandy soil, independent on the fertilizer used, had always higher amounts of 1,8-cineole (8.8-11.8 %) than camphor (4.4-8.5%). Limonene and verbenone were also in relative high levels (1.0-8.8 % and 3.4-7.9 %, respectively) in the essential oils of rosemary maintained in different growing media and collected at different harvesting periods.

The harvesting period of the plant material seems to influence the levels of some components of the essential oils. Excepting those samples in which there is no essential oil, due to the lack of plant material in enough amount for the oil isolation, and the oils isolated from plants maintained in the non-fertilized peat fertirrigated with the fertilizer F2, the myrcene content decreased from October to January or even April, from which a new increase was detected (Tables 1 and 2). Although a similar behavior had been observed for α-pinene, some exceptions were observed for the oils isolated from the plants maintained in the non-fertilized and fertilized peat fertirrigated with the fertilizer F2. For the first case, a slight increase of the amounts of α-pinene was detected while for the second case; an increase of this component was registered from October to January and from April to June (Table 2).

The highest levels of 1,8-cineole (8.8-12.2 %) were observed in April or June. For verbenone, the lowest amounts (3.4-5.3 %) were detected during January and April. The highest percentage of this component was detected in the essential oils isolated from the plants maintained in the fertilized peat and fertirrigated with the fertilizer F2, in October.

4 Discussion

The oil yields were in accordance with the literature for this species [4,5,10] as well as their variations according to the harvest time [11,12]. The best oil yields found in June, is coincident with the rise in temperature (Figure 1) and longer photoperiod (Figure 2). According to some authors [11] these results reveal that summer is the best time to exploit rosemary oils. Our results, in spite of a lack of statistic analysis, were also comparable with those obtained by Moretti *et al*. [13] who observed the agronomic treatments tested did not greatly affect the rosemary yields in contrast to the harvesting time.

The highest percentage of myrcene present in the essential oils of rosemary are also in accordance with the literature in which the essential oils of Portuguese *R. officinalis* are referred as belonging to the myrcene type [1,14] in contrast to the oil samples from Hungary, Spain and Italy [4,10], that were rich in α-pinene, 1,8-cineole and camphor, and those from Algeria mainly constituted by 1,8-cineole [5]. The quantitative differences of myrcene in the rosemary oils detected in the present experiment and depending on the harvest time were also reported by Lawrence [14], though the data only referred to the flowering stages. Despite quantitative variations of α-pinene, 1,8-cineole and camphor had been detected by some authors for this species in other countries [7,11,15] depending on the collection period, our own results seem also to suggest that such variation, in some instances, also depended on the agronomic treatments. These factors induced different plant growths, not quantified in the present work, but verified as producing insufficient biomass for the oil isolation in some growing media as well as in some fertirrigations used. Some authors [11] also detected different macroscopic signs of the rosemary plants as well as quantitative differences in some oil components according to the soil types though such variations were also dependent on the collection period.

Verbenone, an important component in high quality essential oils of *R. officinalis* L. [16], was also in relative high percentages in the oils, depending on the harvesting period. Similar behavior was observed by some authors [11] in rosemary plants maintained either in a granitic silt soil or in a highly calcareous soil. It is noteworthy to refer that the percentages of verbenone found in the rosemary essential oils also depend on its geographic origin 1,6,7,10]. The percentages of verbenone seem also to depend on the oil isolation type because the levels of this component found by Miguel *et al*. [17], using a distillation-extraction procedure, were superior, exceeding in the most cases 10 %. Therefore, the influence of the isolation procedure on the chemical composition of the essential oils should also be kept always in mind.

5 Conclusion

The different stages of the plant life cycle reveal to be important either on the yield or the chemical composition of the rosemary oils, mainly in the major components. Nevertheless, at this stage of our investigation, the predominant influence of the growing media and fertilizer used in the experiment on the oil quality remains to clarify.

Acknowledgments

The authors would like to acknowledge the financial support from FCT (Portugal) and CDCTPV (Portugal) for this work.

References:

[1] Pintore, G., Usai, M., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., Chessa, M., Cerri, R., Casanova, J., Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica, *Flavour and Fragrance Journal*, 7, 2002, 15-19.

[2] Dellacassa, E., Lorenzo, D., Moyna, P., Frizzo, C. D., Serafini, L. A., Dugo, P., *Rosmarinus officinalis* L. (Labiatae) essential oils from the South of Brazil and Uruguay, *Journal of Essential Oil Research*, 11, 1999, 27-30.

[3] Porte, A., Godoy, R. L. O., Lopes, D, Koketsu, M., Gonçalves, S. L., Torquilho, H. S., Essential oil of *Rosmarinus officinalis* L. (rosemary) from Rio de Janeiro, Brazil, *Journal of Essential Oil Research*, 12, 2000, 577-580.

[4] Arnold, N., Valentini, G., Bellomaria, B., Hocine, L., Comparative study of the essential oils from *Rosmarinus eriocalyx* Jordan & Fourr. from Algeria and *R. officinalis* L. from other countries, *Journal of Essential Oil Research*, 9, 1997, 167-175.

[5] Boutekedjiret, R., Bentahar, F., C., Bellabes, Bessiere, J. M., The essential oil from *Rosmarinus officinalis* L. in Algeria, *Journal of Essential Oil Research*, 10, 1998, 680-682.

[6] Tuberoso, C. I. G., Satta, M., Cabras, P., Garau, V. L., Chemical composition of *Rosmarinus officinalis* oils of Sardinia, *Journal of Essential Oil Research*, 10, 1998, 660-664.

[7] Elamrani, A., Zrira, S., Benjilali, B., A study of Moroccan rosemary oils, *Journal of Essential Oil Research*, 12, 2000, 487-495.

[8] Diab, Y., Auezova, L., Chebib, H., Chalchat, J. C., Figueredo, G., Chemical composition of Lebanese rosemary (*Rosmarinus officinalis* L.) essential oil as a function of the geographical region and the harvest time, *Journal of Essential Oil Research*, 14, 2002, 449-452.

[9] Anonymous, *European Pharmacopoeia*, 3rd edn. Strasbourg, Council of Europe, pp. 121-122, 1996.

[10] Domokos, J., Héthelyi, E., Pálinkás, J., Szirmai, S., Tulok, M. H., Essential oil of rosemary (*Rosmarinus officinalis* L.) of Hungarian origin, *Journal of Essential Oil Research*, 9, 1997, 41-45.

[11] Moretti, M. D. L., Peana, A. T., Passino, G. S., Solinas, V., Effects of soil properties on yield and composition of *Rosmarinus officinalis* essential oil, *Journal of Essential Oil Research*, 10, 1998, 261-267.

[12] Serrano, E., Palma, J., Tinoco, T., Venâncio, F., Martins, A., Evaluation of the essential oils of rosemary (*Rosmarinus officinalis* L.) from different zones of "Alentejo" (Portugal), *Journal of Essential Oil Research*, 14, 2002, 87-92.

[13] Moretti, M. D. L., Peana, A. T., Passino, G. S., Bazzoni, A., Solinas, V., Effects of iron on yield and composition of *Rosmarinus officinalis* essential oil, *Journal of Essential Oil Research*, 10, 1998, 43-49.

[14] Lawrence, B. M., Progress in essential oils, Perfumery Flavorist, 14, 1989, 49-54.

[15] Boutekedjiret, C., Bellabes, R., Bentahar, F., Bessiere, J. M., Study of *Rosmarinus officinalis* L. essential oil yield and composition as a function of the plant life cycle, *Journal of Essential Oil Research*, 11, 1999, 238-240.

[16] Ravid, U., Putievsky, E., Katzir, I., Lewinsohn, E., Dudai, N., Identification of (1*R*)(+)-verbenone in essential oils of *Rosmarinus officinalis* L., *Flavour and Fragrance Journal*, 12, 1997, 109-112.

[17] Miguel, G., Guerrero, C., Rodrigues, H., Brito, J., Venâncio, F., Tavares, R., Martins, A., Duarte, F., Study of the substrate and fertilisation effects on the production of essential oils of *Rosmarinus officinalis L.* cultivated in pots. In Anaç D., Martin-Prével, P. (Eds.), *Improved Crop Quality by Nutrient Management*, Kluwer Acad Publishers, Netherlands, pp. 185-188, 1999.

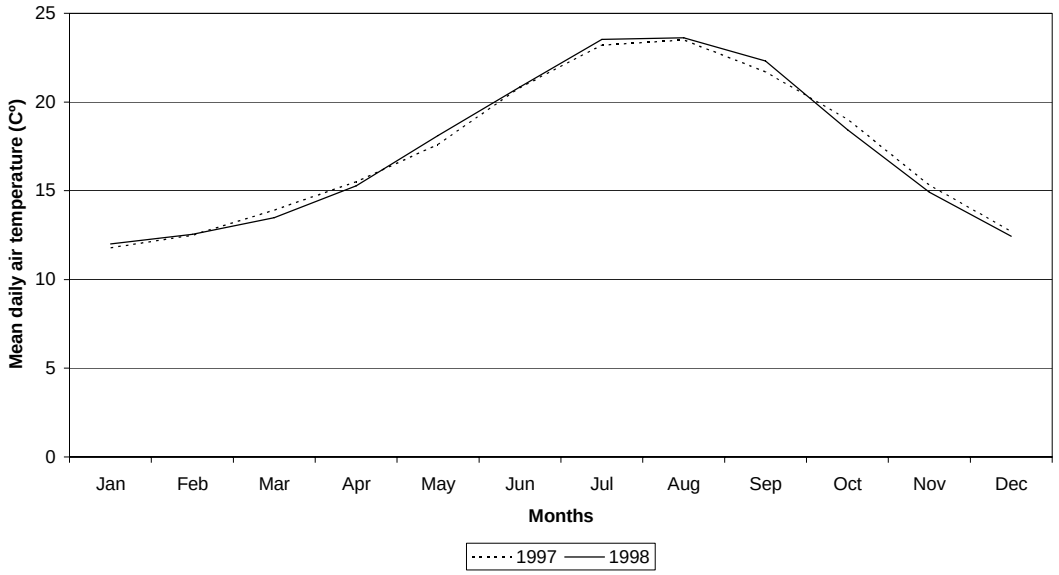

Figure 1. Mean daily air temperature during the experimental period.

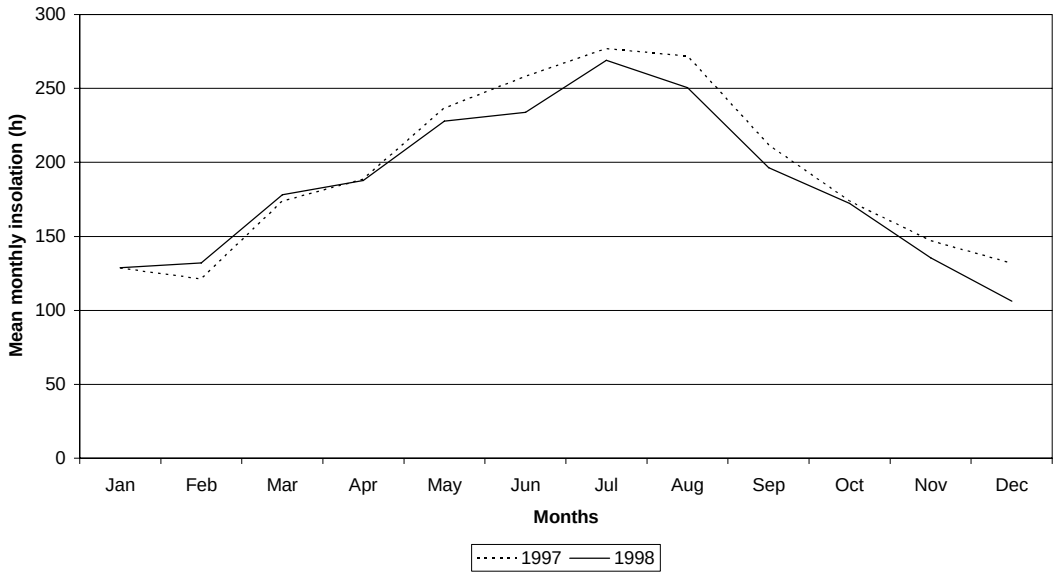

Figure 2. Mean monthly insolation hours during the experimental period.

Table I. Oil yield and percentage composition of the essential oils isolated from the aerial parts of *Rosmarinus officinalis* L. maintained in three growing materials (local sandy soil-**A**; fertilized peat-**B**, non-fertilized peat-**C**) and fertirrigated with a solution containing 5g.L^{-1} of Hakaphos 15:05:30 with 2% Mg (F1) and collected in different harvesting periods.

	October	January	April	June	Oct.	January	April	June	October	January	April	June
Compound	**A**	**A**	**A**	**A**	**B**	**B**	**B**	**B**	**C**	**C**	**C**	**C**
α-Pinene	15.4	13.1	12.8	15.8		15.1	13.4	13.1	16.5	15.5	11.8	12.3
Camphene	3.1	3.7	3.8	4.0		4.6	3.8	3.6	3.1	3.8	3.3	3.3
β-Pinene	3.9	5.8	6.6	4.6		4.7	7.3	3.7	3.6	5.1	6.5	4.2
Myrcene	30.0	25.8	23.2	25.9		21.6	24.6	27.5	29.9	27.7	24.0	29.9
α-Terpinene	1.0	0.7	0.7	0.8		1.0	0.9	0.7	0.8	0.8	0.6	0.7
p-Cymene	0.5	1.0	0.5	1.9		0.6	0.8	1.5	0.4	0.8	0.6	0.8
Limonene	4.8	5.3	6.0	3.6		1.0	5.1	5.4	5.0	6.9	5.2	6.8
1,8-Cineole	8.8	10.2	10.7	11.8		10.0	10.6	11.3	8.6	8.6	12.2	8.8
cis-Ocimene	2.4	2.8	1.6	3.6		1.7	3.8	2.4	0.6	1.0	1.1	1.0
γ-Terpinene	3.2	3.8	3.0	2.7		2.4	3.7	2.5	2.2	2.9	2.4	2.1
Terpinolene	1.2	1.1	1.0	0.9		1.1	1.0	0.9	1.2	1.2	1.0	0.9
Linalool	1.3	1.0	1.1	1.0		1.2	1.0	1.6	1.8	1.1	1.1	1.4
Camphor	4.4	7.6	8.5	7.4		11.0	7.4	9.4	9.2	8.9	9.8	12.2
Borneol	1.0	1.4	1.3	1.1		1.0	1.4	1.0	1.1	1.1	0.7	1.1
Terpinen-4-ol	1.0	1.1	1.3	1.1		1.0	1.2	1.1	0.9	0.7	1.1	1.0
α-Terpineol	1.4	1.3	1.6	1.3		1.4	1.5	1.5	1.4	1.1	1.5	1.5
Verbenone	5.8	4.0	3.6	4.8		4.0	4.2	6.4	5.3	3.5	3.5	5.8
βCaryophyllene	1.1	2.0	3.1	0.8		2.0	1.8	0.7	1.0	1.4	3.0	0.5
Caryophyllene oxide	0.4	1.0	1.0	0.6		1.1	0.7	0.5	0.3	0.5	1.3	0.5
Oil yield (v/w)	0.5	0.4	1.2	1.3		0.5	1.0	1.4	0.5	0.6	0.7	1.4

Table II. Oil yield and percentage composition of the essential oils isolated from the aerial parts of *Rosmarinus officinalis* L. maintained in three growing materials (local sandy soil-**A**; fertilized peat-**B**, non-fertilized peat-**C**) and fertirrigated with a solution containing Hakaphos 13:40:13 with 0.4% Mg (F2) and collected in different harvesting periods

	October	January	April	June	October	January	April	June	October	January	April	June
Compound	**A**	**A**	**A**	**A**	**B**	**B**	**B**	**B**	**C**	**C**	**C**	**C**
α-Pinene	15.4	15.0	13.4		8.8	14.6	12.2	14.4	11.8	12.3	12.6	
Camphene	3.5	4.2	3.7		2.5	4.0	3.8	3.8	2.4	3.0	3.5	
β-Pinene	3.7	5.7	7.4		2.0	4.2	6.3	4.3	3.2	5.1	5.8	
Myrcene	26.1	22.8	23.4		28.5	26.0	22.8	24.6	22.2	28.6	24.0	
α-Terpinene	0.8	0.8	0.7		0.6	0.9	0.7	0.6	0.4	0.7	0.6	
p-Cymene	0.6	1.0	0.6		1.1	1.0	0.8	1.2	0.8	0.6	0.5	
Limonene	5.5	5.0	6.8		6.4	7.0	7.1	8.8	7.9	8.2	7.2	
1,8-Cineole	9.6	10.7	9.5		9.6	9.0	10.8	10.0	8.0	8.8	11.4	
cis-Ocimene	2.6	2.7	1.8		0.6	2.2	1.2	1.1	0.6	1.0	1.0	
γ-Terpinene	2.7	3.0	2.7		1.8	2.9	2.7	2.6	2.0	2.8	2.1	
Terpinolene	1.2	1.0	0.8		1.2	1.1	1.1	1.4	1.3	1.1	1.2	
Linalool	1.9	0.7	1.2		3.5	1.2	1.4	1.3	2.2	0.9	1.1	
Camphor	6.6	7.5	7.4		14.1	8.0	10.5	9.2	11.8	10.1	10.3	
Borneol	1.1	1.2	1.4		1.4	1.1	0.6	1.0	1.1	1.1	1.0	
Terpinen-4-ol	1.1	1.0	1.0		1.2	1.0	1.1	0.9	1.1	0.9	1.0	
α-Terpineol	1.4	1.2	1.5		1.9	1.3	1.5	1.3	1.5	1.4	1.6	
Verbenone	6.7	4.6	3.9		7.9	5.3	5.1	4.5	4.8	3.4	4.6	
βCaryophyllene	1.4	1.8	2.8		0.7	1.1	2.1	1.4	2.5	0.8	2.7	
Caryophyllene oxide	0.4	t	0.7		0.6	0.6	1.2	0.6	1.1	0.6	1.2	
Oil yield (v/w)	0.5	0.3	0.9		0.6	0.6	1.0	1.0	0.7	0.7	0.7	