EXTRACTION AND IMPREGNATION OF LAVENDER ESSENTIAL OIL IN HDPE USING SUPERCRITICAL CARBON DIOXIDE

[a]Anita Lourenço, [a]Pedro Lisboa, [a]Alexandre Paiva, [b]António Soares, [c]Marco Miranda, [c]Carla Rodrigues, [a]Ana Aguiar-Ricardo, [a]Pedro Simões and [a]Teresa Casimiro*

[a]REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia,
Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.
[b]BeyonDevices Lda, Alcabideche, Portugal
[c]NOVADELTA S.A., Herdade das Argamassas, 7370-179 Campo Maior, Portugal
*e-mail: teresa.casimiro@fct.unl.pt

ABSTRACT

The aim of this work is to explore the potentialities of loading high density polyethylene (HDPE) devices, a polymer typically used in biomedical applications, with an essential oil using supercritical carbon dioxide ($scCO_2$). The objective is to provide valuable new properties for the end user of such devices such as fragrance release and reduction of surface biofouling formation by microorganisms during usage. The work consisted mainly in three parts: 1) supercritical fluid extraction and chemical profiling of the essential oil from lavender plants, *lavandula angustifolia,* harvested in Alentejo, Portugal. The extracted oil was then analysed by GC-MS and compared with the one obtained by hydrodistillation; 2) $scCO_2$-assisted impregnation of the oil in the HDPE samples; 3) Assessment of biocompatibility and anti-biofouling activity of the impregnated HDPE samples against *Candida albicans*.

INTRODUCTION

Polyethylene (PE) is considered the most important and extensively used thermoplastic because of its low cost, good processability and diverse range of applications [1]. Biologically anti-biofouling properties are of great importance since PE is widely used in the development of biomedical materials such as catheters or sutures [2]. Biofouling of proteins, cells, or bacteria on the surface of biomedical devices and implants is a healthcare problem and requires, in some cases, painkiller and antibiotic treatment, influencing negatively the quality of life of patients. Thus, there is a need for solutions that prevent infections, avoiding biofouling, and making the device surface undetectable by proteins or microorganisms in a biological environment [3].

Lavender oil is well known for its application in aromatherapy, cosmetics, soaps and perfumes [4]. Some cases of activity against bacteria and fungi have been reported [5], which incentive its application in antifouling and antimicrobial materials.

Supercritical fluids (SCFs) have been recognized as excellent extraction solvents, with special attention given to supercritical carbon dioxide ($ScCO_2$) due its lower critical pressure and temperature, availability and safety being considered as a GRAS solvent. Such advantages combined with the enhanced gas-like transport properties at the supercritical region makes the

$scCO_2$ a solvent of choice in the food and fragrances industry proven by the coffee decaffeination, hop and essential oil extraction [6]. Besides extraction, $scCO_2$ is also successfully applied as a reaction medium in polymer synthesis [7], impregnation [8] and purification [9].

MATERIALS AND METHODS

Supercritical fluid extraction

Fresh lavender was harvested in the south of Portugal, Alentejo at the second harvest time in mid-October. The aerial parts were dried at room temperature for at least 20 days. The flowers were separated from steam and leaves and were stored at -20ºC prior to extraction. The supercritical extraction experiments were carried out in a laboratory scale unit equipped with a 100 cm^3 capacity high-pressure vessel. About 20g of flowers where loaded into the vessel and the carbon dioxide (99.998 % purity) was pressurized by means of a Williams air driven pump while the pressure was controlled by a back pressure relief valve and the temperature by electrical heating tapes. The extraction experiments were performed at 9 MPa and 45ºC [10] with a solvent mass flow rate of 1.5 g/min for 120 min. The extracted oil was collected by depressurization into a trap immersed on ice.

The same amount of flowers was used for the hydrodestillation in a Clevenger type apparatus using 500 ml of deionized water for 3h hours accordingly to the European Pharmacopoeia [11]. The oils were sealed and stored at 8ºC. The oil analysis was performed in a Bruker Scion GC/MS equipment using a ZB-WAX® column(20m×0.25mm), according with a method reported elsewhere [12].

Supercritical impregnation of lavender oil

Polyethylene was cut into similar pieces and impregnated with lavender oil in $scCO_2$ using a high-pressure cell. The impregnation was performed at 115 ºC and 180 bar using an excess of oil. After 20 h of continuous stirring the high pressure vessel was rapidly depressurized.

Growth of *C. albicans*

C. albicans strain PYCC was regrown from frozen stocks. For experiments, YMB (5 ml) was inoculated with one colony of Candida overnight. After that time, the suspension was diluted to 10^5 cells in YMB medium.

Viability of yeast cells on the surface

Impregnated polymer samples (~25 mg) were placed in the wells of a 24-well tissue culture plate with 1ml of medium and 5µl of the microorganism suspension (10^5 cells), and incubated at 37°C with shaking for 48h.

Biocompatibility Studies

Human fibroblast cells were seeded, in contact with sterilized PE samples, at a density of $4x10^4$cells/well and cultured with DMEM-F12. Subsequently, the mitochondrial redox activity of viable cells was assessed through a resazurin assay (n=5). At 24 and 72 hours, cells were incubated with 100 µL DMEM-F12 and 10 µL of resazurin 0.1% (w/v) in 5% CO_2 humidified incubator, for 24h, at 37ºC. Fluorescence of metabolized resazurin was measured using a Gemini EM spectrophotometer at an excitation/emission wavelength of λ=545/590nm,

respectively. Wells containing cells in the neat culture medium were used as positive control. Ethanol (96%) was added to wells that were used as a negative control.

RESULTS

Supercritical fluid extraction and hydrodistillation of lavender plants gave similar results in terms of oil yield, about 3.4 % (mass of oil/dry lavender flowers). The main compounds of the oil were found to be cis-β-ocimene, linalool, linalyl acetate, β-caryophyllen and lavandulyl acetate. Surprisingly, camphor, 1,2-cineole and α-pinene, compounds often related to antifungal and antimicrobial properties of the lavender oils were only present in trace amounts, less than 0.1%. The major compounds of the essential oil are depicted in table I.

Table 1 Composition of Lavender essential oil obtained by $C0_2$ and water based extraction methods. (% calculated by area integration).

Compound	$scCO_2$ extraction	Hydrodistillation
Trans-β-ocimene	3.99	5.31
Cis-β-ocimene	5.80	8.15
α-ocimene	1.28	1.71
1-octen-3-yl acetate	2.84	3.14
Linalool	15.99	27.32
Linalyl acetate	36.02	17.34
Farnesene	0.19	<0.1
β-Caryophyllen	6.10	1.91
α-terpeniol	1.17	1.31
Lavandulyl acetate	5.86	5.92
Germancrene	3.41	1.29
Lavandulol	1.00	0.96
Fenchol	<0.1	5.16
Borneol	0.86	<0.1

Significant differences between both methods were observed particularly in the composition of linalool and linalyl acetate. The oil obtained by the $scCO_2$ extraction method contained higher amounts of linalyl acetate and lower percentage of linalool. This can be explained, as expected, by the different affinity of each compound for the solvent of extraction. However, in hydrodistillation this trend was not observed as a consequence of the partial hydrolysis of linalyl acetate into linalool, as also observed in literature [10].

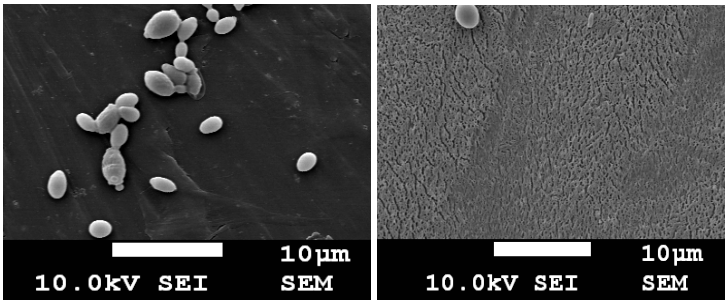

Figure 1: SEM images of PE without (left) and with (right) extracted oil, incubated with *C. albicans* for 48 h.

The viability of yeast cells on the surface of the impregnated PE samples was assessed by incubating them in medium containing *C. albicans* for 48 h. Comparison of scanning electron micrographs (SEM) of the polyethylene samples surfaces, with and without the extracted oil (Fig. 1) showed that the presence of oil dramatically reduced the fungi at the surface.

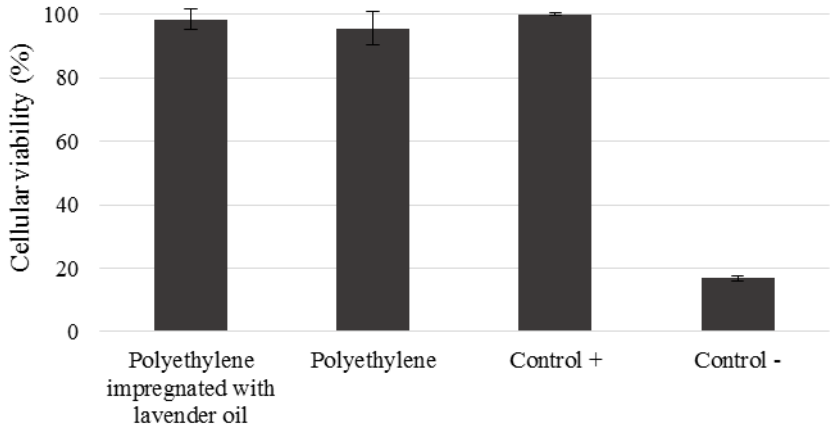

Figure 2: Cellular activity by resazurin test after 24 h.

The biocompatibility of the polyethylene impregnated with lavender oil was accessed by resazurin test (fig. 2). The results show that the polyethylene impregnated with oil presents a cellular viability slightly higher than neat polyethylene, near to the positive control, evidencing complete biocompatibility of the material.

CONCLUSION

Lavender can be successfully extracted and easily impregnated into PE samples using supercritical technology. The preliminary results obtained show that the impregnation of lavender essential oil into PE devices is very promising in terms of anti-biofouling activity and biocompatibility, suggesting that the oil could be used in biomedical devices.

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REFERENCES

[1] AKIYAMA, S.; INOUE, T.; NISHI, T. In Polymer Blend, **1981**, p. 56

[2] LAVANANT, L., et al. Macromolecular bioscience, 10, **2010**, p. 101

[3] BANERJEE, I.; PANGULE, R. C.; KANE, R. S. Advanced Materials, 23, **2011**, p. 690

[4] LIS-BALCHIN, M., The genus Lavandula, **2001**

[5] DEANS, S. G., RITCHIE, G., Int. J. Food Microbiol. 5, **1987**, p.165

[6] REVERCHON, E., J. Supercrit. Fluids. 10, **1997**, p.1

[7] COOPER, A. I., Journal of Materials Chemistry, 10, **2000**, p. 207

[8] MUTH, O.; HIRTH, T.; VOGEL, H., The Journal of Supercritical Fluids, 17, **2000**, p. 65

[9] DÍAZ-REINOSO, B., et al. Journal of agricultural and food chemistry, 54, **2006**, p. 2441

[10] REVERCHON, E., DELLA PORTA, G., J. Agric. Food Chem., **1995**, p.1654

[11] Council of Europe European (COE), European Directorate for the Quality of Medicines (EDQM), European Pharmacopoeia (6th ed.), **2007**

[12] KIM, N., LEE, D., J. Chromatogr. A. 982, **2002**, p.31