Encapsulation of the essential oil of *Eucalyptus Globulus* by adsorption and coating in fluidised bed

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1. Introduction

Use of essential oil (EO) is the subject matter of many investigations for in recent years due to its éco-friendly and biodegradable nature (Maji et *al.,* 2007). Among the essential oils, eucalyptus oil is of special interest for its strong in vitro antibacterial activity against human and animal pathogen microorganisms (Chih et Toshiaski, 2003). It was reported as alternative to chemical animal antibiotic growth promoters (Robert, 2005). In Concentrated Powder Form (CPF) technology already used in spices and animal feed industries, the liquid is either adsorbed by the solid surface or absorbed in the cavities of porous carriers (Lankes et *al.,* 2003). However, the liquid active substance remained sensitive to degradation and volatilisation (Savitha et *al.,* 2005). To avoid that, the elaboration of a "physical" barrier between the sensitive ingredient and the environment which is the main objective of encapsulation can be achieved (Fuchs et *al.,* 2005).
The present work deals with innovative encapsulated liquid ingredients powder forms either adsorbed by the solid surface or absorbed in the cavities of porous carriers. In its simplest formulation, the solid phase is made of an adsorbent microparticles and oil incorporation is achieved using simple mixing process. Oil/adsorbent system is then coated in fluidised bed.
The purpose of this study was to test de feasibility of the process and investigate the following properties of the microcapsule obtained: (i) to evaluate the efficiency of microencapsulation and analyse the microstructure and particle size distribution of fluid bed encapsulated products; (ii) to assess the changes in the composition of flavours taking place during processing; and (iii) to measure the release of volatiles from microencapsulated products.

2. Materials and methods

2.1. Materials

Adsorbent materials used here to form oil/adsorbent core materials included respectively white spherical beads of sipernat® 22 (Degussa AG, Germany) and fujicalin® (Fuji Chimical Industry Co., Ltd.), with a mean particle diameter of 110µm (resp 110µm) and a spécific surface area of 190m^2/g (resp 40m^2/g). For coating, a 30% (w/w) aqueous solution of Arabic gum was used as a coating solution. Dyes were added to the aqueous solution. This precaution is necessary for identified the envelope around the microparticules. A lab-scale startic agitated reactor was used for adsorption and the classical lab-scale Wurster (a bottom-spray coater) UNIGLATT (Glatt, Binzen) was used for aqueous coating.

2.2. Experimental procedure

150g of adsorbent bed materials contained in the developing tank of RSA was fluidised with the 1200tr/min rotating rod of magnetic agitator of RSA. The EO was then spray in the top of agitated bed until desired adsorption capacity. Immediately after, oil/adsorbent system is transfer to the fluid bed chamber. For coating, 30%(w/w) aqueous solution was sprayed at a rate of 12,5g/min through the nozzle in the fluid bed chamber for coating. The atomization pressure and air agitation was respectively set to 20 bars and 90%. The temperature of the inlet was varies from 30°C to 60°C. The experiment was run for the necessary time to reach the coating level of 20% and obtained dry powder.

2.3. Particles analyses

The Encapsulation Efficiency (EE) was calculated as the ratio between the initial mass of EO to be encapsulated and its mass in the final product. It was obtained by evaporating total humidity and EO of about 5g weighed microcapsules sample using infrared thermo-gravimetric humidimeter (Mettler Toledo, France).

EO mass was determined by subtracting the mass of humidity obtained from blanc samples, processed in the same conditions witout EO. The **external and internal structures** of microcapsules were evaluated with optical microscope (OM), (ZEISS) and SEM (Hitachi S2500 scanning electron microscope (Hitachi Science Systems Ltd., Ibaraki, Japan)). The powders were placed on the SEM stubs using a two-sided adhesive tape and then analyzed at 12 kV acceleration voltage after Pt–Pd sputtering by MSP-1S magnetron sputter coater (Vacuum Device, Tokyo, Japan). Examination was made at 100, 300 1000 and 3000 magnifications. (Rénata et al., 2006). **Diameter of microcapsules** was determinated using a laser diffraction-based Malvern particle size analyser Mastersizer S fitted with an MSX64dry powder feeder unit-sample (Malvern Instruments Inc.,UK) (Rénata et *al.,* 2006). **The heat stability** of the microcapsules was estimated by measuring the time course of the weight Wm(t) of the microcapsules (approximately 5 g of particles) placed at 70 °C in the oven commonly used to determine the water content of samples. Weights of the sample were measured continuously and recorded mannualy using a balance. The oil release content was defined as $\psi(\%)=[(W_m-W_m(t))/(W_m-W_0)]\times100$, where W_0 denotes the weight of microcapsules measured after complete evaporation of EO at 120°C for 3 h (Chih et *al.,* 2006). **Identification of EO compounds** was performed with a Hewlett Packard 6890 chromatograph linked to a Hewlett Packard 5973 mass spectrometer system operating on EI mode (equipped with a split/ splitless injector (200°C) 1/10 split ratio), and a 30 m long, 0.25 mm i.d., 0.25 mm film thickness HP 5MS capillary column. The ionization energy was 70 eV. The temperature of the injection block was 200°C. The GC oven temperature was programmed as follows: initial temperature 60°C (for 5 min) followed by a temperature increase of 3°C min^{-1} up to 280°C. The carrier gas was He. Identification of the volatile components was established using a Wiley 6 MS data library and GC–MS data with those of authentic samples (Andreas et *al.,* 2001).

3. Results and discussion

3.1. Effect of technological parameters on Humidity, EE, and diameter of microparticules.

The type of adsorbent, AC, Ti, and on EE, particles sizes and moisture content were investigated as shown in Table 1.

N°	Produit	T_i(°C)	AC (%)	Humidity (%)	EE (%)	D (µm)
1	fujicalin	30.00	60	2.0±0.0 ^{a}	21.0±0.0 ^{b}	320.64±15.00 ^{e}
2	fujicalin	50.00	60	2.0±0.0 ^{a}	5.0±0.0 ^{a}	274,84±5.13 ^{d}
3	sipernat® 22	30.00	60	19.4±1.7 ^{b}	93.4±2.6 ^{f}	144.93±1.02 ^{a}
4	sipernat® 22	30.00	160	20.0±0.0 ^{b}	67.0±7.13 ^{c}	163.33±8.07 ^{bc}
5	sipernat® 22	40.00	60	23.0±0.0 ^{c}	98.0±0.0 ^{f}	142.96±1.21 ^{a}
6	sipernat® 22	50.00	60	18.5±0.7 ^{b}	92.5±2.1 ^{f}	142.11±2.83 ^{a}
7	sipernat® 22	50.00	160	18.7±0.6 ^{b}	76,7±3.6 ^{cde}	176.42±3.41 ^{c}
8	sipernat®22	60.00	60	17.5±0.7 ^{b}	74.0±3.5 ^{cd}	145.74±6.043

a, b, c, d, e, f : significant difference at 95%

Table 1: Characteristics of the microcapsules obtained at different technological conditions

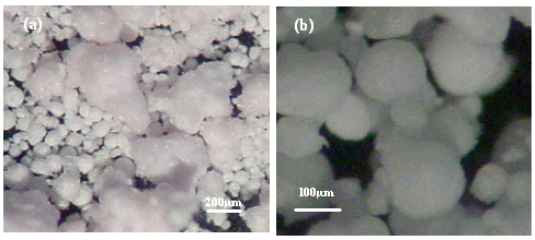

Fig. 1: OM microphotography of microcapsules (a) fujicalin (b) sipernat® 22

3.1.1. Effect of type of adsorbent (*run 1-3, 6*): Fujicalin offer powders with low EE, low humidity and high mean diameters than sipernat® 22. The reason may be associated with agglomeration of fujicalin observed during coating (Fig 1a). This lead to humid block which take long time to be dried. The phenomenon is not happened with sipernat® 22 (Fig 1b) which is already used as anticaking, and flow improvement behaviour (Fuchs et *al.,* 2006).

3.1.2. Effect of AC (*run 3, 4, 6 and 7*): When AC increase, the EE decreased. This indicates that the EE depend on the quantity of oil loaded in the adsorbent and can be due to rapid evaporation in fluid bed of weak strength bounded oil in the surface of adsorbent with high AC. The increasing of microcapsule size with AC may be explained by the increasing of sipernat® 22 adsorbent pores

3.1.5.Effect of inlet temperature (*runs 1, 2, 3, 5, 6, 8*):Increasing inlet temperature from 30 to 60 °C while keeping other parameters unchanged, both EE and humidity increased not significantly at the beginning and reached a high value of 98.0% and 23%, at the inlet temperature reached 40°C. When inlet temperature increased to 60°C, both Humidity and EE were significantly decreased. Increasing inlet temperature led to high evaporation of EO of adsorbent and Water of emulsions of coating solution, which may result low EE and humidity. Therefore, the optimal technological parameters were determined as feed temperature of 40°C. There is not significant effect of Ti in the diameter of microparticules.

3.2. Heat stability

Fig. 2 showed the storage stability of the coated sipernat® 22 with EO and uncoated sipernat® 22 with EO. If the experiment data were placed into the exponential function $\psi(t) = C\text{eq}(1-\exp -t/\tau)$ (Chich, 2003) to generate the solid line in the figure, the results are generally consistent with values obtained from the experiment. Thus, the oil release curves are fitted well to the exponential function as aforementioned. It demonstrated that the analysis values are reliable because all the correlation coefficient in the exponential function are greater than 0.99, where *C*eq means the concentration of oil released at the equilibrium state, τ as the time constant, t as the time release. As the results shown in table 2 coating oil/adsorbent microparticles system would increase the value of τ. Thus, the results indicate that the release rate during heating is reduced by coating.

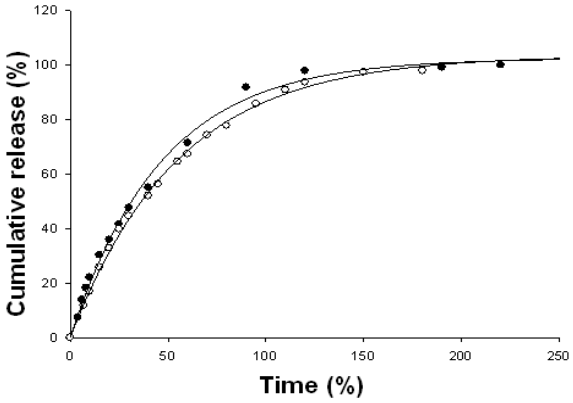

Fig 2 : EO libération curve of microparticules with time at 70°C. [(○) coated sipernat 22®.AC 60% (●) uncoated curves sipernat 22®. AC 60%]

Produit	τ (min)	R^2
Coated sipernat 22®	55,56±1,52[b]	0,99±0,00
Uncoated sipernat 22®	48,31±1,04[a]	0,99±0,00

Table 2: Time constant of EO liberation with time at 70°C

3.2. Identification of volatile

TR		Identification	Crude oil	sipernat 22®.Fujicalin	
		Noms	*Area (%)*	*Area (%)*	*Area (%)*
7,001	MS	dl-limoneme	0.06	-	-
7,204	MS	α-thujene	0.12	-	-
7,56	**MS**	**α-pinene**	**9.70**	**2.82**	**0.60**
9,52	MS	2-β-pinene	0.45	0.22	0.04
10,076	MS	β-myrcene	0.91	0.72	0.22
10,89	MS	1-phellandrene	0.93	0.73	0.20
11,422	MS	α-terpinene	0.24	0.20	0.05
12,582	**MS**	**1,8 cinéole**	**79.66**	**85.82**	**67.45**
12,946	MS	delta,3-carene	0.23	0.28	0.08
13,551	**MS**	**γ-terpinene**	**4.62**	**5.17**	**3.70**
14,159	MS	trans linalool oxide	0.07	0.09	0.11
14,851	MS	α-therpinolene	0.28	0.35	0.29
15,101	MS	para-cymene	0.05	0.06	0.04
15,642	MS	l-linalool	0.15	0.19	0.88
16,609	MS	D-fenchyl alcool	0.03	0.04	0.13
17,048	MS	Allocimene	0.09	0.12	0.05
17,684	MS	trans-pinocarvéol	0.14	0.19	0.96
19,206	MS	delta-terpineol	0.09	0.10	0.82
19,716	MS	3-cyclohexen-1-ol	0.47	0.66	3.19
20,074	MS	1(7),3,8-o-menthatriene	0.02	0.03	0.28
20,484	**MS**	**α-terpinéol**	**1.42**	**1.66**	**15.01**
20,698	MS	Camphene	0.02	0.03	0.28
23,181	MS	Geraniol	0.03	0.04	0.53
27,744	MS	α-terpinenyl acetate	0.12	0.23	0.96
30,482	MS	α-gurjunene	0.02	0.04	0.10
31,865	MS	aromadendrene	0.07	0.14	0.44

TR: Retention time

Table 3: Composition of EO before and after encapsulation in GC area percent

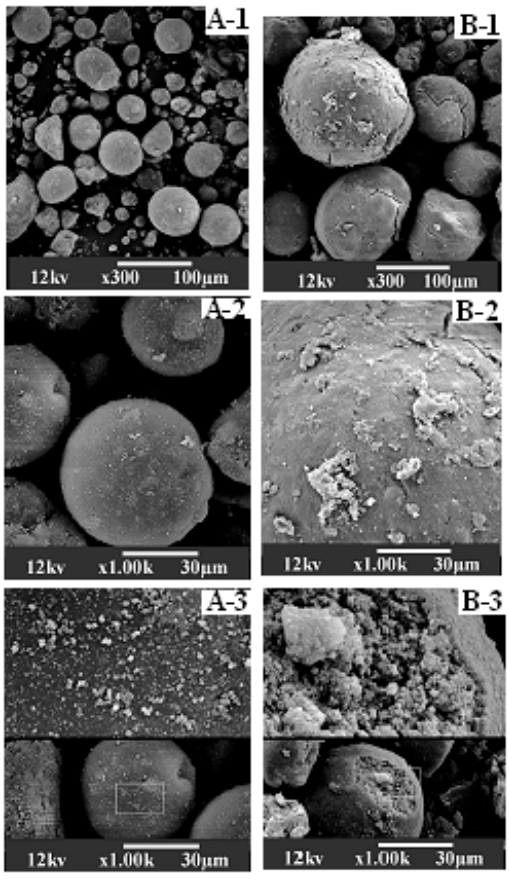

Fig. 3: SEM imagesof microcapsules A)- uncoated sipernat® 22 with 0% AC B)- coated sipernat® 22 at 30°C with 60% AC

When natural flavorings are encapsulated in the different matrixes it is important to know the changes in the composition taking place during encapsulation process. Such knowledge is useful in designing preparations with a specified flavoring and/or antimicrobial activity. The composition of pure and microencapsulated *Eucalytus* EO in sipernat 22® and Fujicalin are reported in table 3. The composition of pure and microencapsulated EO was quite similar. However, some changes in the percentage of some individual compounds are observed (in bold). For instance, the content of α-pinene in sipernat 22® (2.82%) and Fujicalin (0.60%) microcapsules was lower than in pure EO (9.70%). This result could be explained by losses during fluidisation. The consequent is the increasing of the amount of 1.8 cineol in sipernat 22®. In fujicalin both decrease of 1.8 cineol and increase of α-terpineol are observed. This can be explained by the possible conversion previously reported by (Williams, 1998)

3.3. Microstructural properties of fuid bed microencapsulated particles by SEM

SEM was used to investigate external–internal structure of encapsulated EO in the matrices. Fig. 3 A (A-1, A-2, and A-3), and B (B-1, B-2, B-3) show respectively the SEM picture of uncoated sipernat 22® without EO and coated sipernat 22® with EO (60%AC). The shape of each powder was almost the same for each category (uncoated and coated). This is normal because resulting fluid bed microcapsules usually take de shape of adsorbent used. Wall matrice was observed in the surface of coated microcapsules (Fig 3 B-2). This showed the effectiveness of coating. Fig 3 B-3 showed the internal porous shell of microcapsule which may contend encapsulated EO. Crack was observed in the external structure of microcapsule. This may be the consequence of collision in fluid bed or in the absorption reactor.

Conclusion

Eucalyptus globulus EO microcapsules were successfully prepared by a adsorption an coating in fluid bed method using a wall system consisting of Arabic gumm. EE were significantly higher in sipernat® 22 adsorbent, and at inlet temperature less or equal to 50°C. Coating reduced the release rate of EO. SEM showed that the microcapsules had a regular spherical shape this study would be helpful to facilitate the used of essential oil in animal food formulation.

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