Lyophilised *Eucalyptus Globulus* coacervates based on β-lactoglobulin and acacia gum

G. H. D. Fendoung[3], M. B. Ngassoum[3]*, C. Sanchez[2] and D. Poncelet[1]
[1] Rue de la Géraudière BP 82225-44322, LGPEA, ENITIAA, Nantes France
[2]2, avenue de la foret de haye BP 172-F-54505, LBB, Vandoeuvre Lès Nancy, France
[3]Department of Applied Chemistry ENSAI University of Ngaoundéré, Cameroon
(Email : guylamda@yahoo.fr)

Introduction

Eucalyptus is one of the world's most important and most widely planted genera. Among its main use is the production of essential oils, which are used for medicinal and pharmaceutical purposes. One of the most important compounds extracted from Eucalyptus is 1,8-cineole, mainly due to its antibacterial and expectorant properties (Giamakis 2001).

In the last decades, micro and nano sized colloidal matrices have received a growing scientific and industrial interest. These vectors may be capsules loaded by living cells, enzymes, flavour oils, pharmaceuticals, vitamins, adhesives, agrochemicals, catalysts and offer considerable advantages at use. Liquids can be handled as solids, odour or taste can be effectively masked in a food product, sensitive substances can be protected from deleterious effects of the surrounding environment and drug delivery can be controlled and targeted (Renard 2002).

One of the main applications of complex coacervation is micro-encapsulation and many articles appeared over the last six years reporting on the characterization of new biopolymer systems for encapsulation purposes in food and pharmaceutical applications The trend in micro-encapsulation nowadays is to replace the traditional gelatin / gum arabic system by new biopolymers, to obtain new capsule properties (de Kruif 2004). Futhermore, the major draw back of the technique is that complex coacervates are highly unstable and that toxic chemical agents, such as glutaraldehyde, are necessary to stabilize them. A major challenge to develop safe multifunctional delivery systems based on complex coacervation will be to find alternative routes to stabilize coacervates (Sanchez 2002).

The aim of this study was to verify the possibility to obtain lyophylised coacervate of eucalyptus globulus essential oil with β-lactoglobulin (BLG) and acacia gum (AG) and explore the potential use of the flavonoid quercetin as stabilising agent in complex coacervation.

The specific objectives were to determine (i) the encapsulation efficiency, (ii) the type and morphology of the microparticules obtained.

Material and Methods

Materials

Eucalyptus globules Essential oil was brought to COOPER company. Arabic gum (AG) was a gift from CNI company (Rouen, France). β-lactoglobulin (BLG) and quercetin came respectively from Davisco Foods International, Inc. (Lesueur, USA) and Sigma-Aldrich (St-Louis, MO USA).

Coacervate preparation

AG, and BLG stock dispersions at total biopolymer concentration of 2 wt% were prepared in deionized water (MilliQ, MilliPore, USA) by gentle mixing. Stock dispersions were left overnight

at 4±1C to ensure good hydration of biopolymers. The pH of the resulting dispersions was adjusted to 4.2 using HCl or NaOH.

Two types of coacervates were prepared at 30°C by adding the polysaccharide solution to protein solution under stirring at protein to polysaccharide weight ratios (Pr:Ps) of 1:1. For the first one, 10% of eucalyptus essential oil, 0.1% of quercetine and 5% of lactose were emulsified in the protein solution. The second one was prepared in the same manner without lactose nor quercetin. The 500g batches were prepared in 1000 ml beakers and the mixtures stirred at level 6 and 12 using a kitchen hand mixer (Braun, MR 6550 CA, France). Before adding the polysaccharide the emulsion was stirred for 3min. After 10min, the water bath was drained and the system was cooled to 4±1°C maintaining gentle mixing with magnetic agitator. The resulting emulsion was then lyophilised.

Structural characterization of particles and encapsulation efficiency

The coacervate ***Size distribution*** was measured by a laser diffraction-based Malvern particle size analyse Mastersizer S fitted with an MSX64 dry powder feed unit-sample (Malvern Instruments Inc., UK) (Baranauskiene 2006)**.** The ***external and internal structures*** of coacervates and dried microcapsules were evaluated using a contrast phase optical microscope Olympus BH-2 (Olympus Optical, Tokyo Japon) 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 x100, x300, x1000 and x3000 magnifications (Baranauskiene 2006). To evaluate the ***encapsulation efficiency*** 0.1g of microcapsules was weighed in a 10ml tube with screwcap and mixed with 4ml pentane. The tube was capped and transferred to a shaking water bath maintained at 90°C for 30min. The supernatant was quantitatively analyzed for flavour compounds using gas chromatography (GC). The supernatant (1µl) was injected into Perichrom (PR 2100, Zac du Moulin, 91160 Saulx-lès-chartreux, France) with a fused silica capillary column (Ultra-2; 50m 0.25mm ID0.2 µm film thickness; (Perichrom)). The linear velocity of the helium carrier gas was 2.0 mL/min. Oven temperature was programmed from 60 to 260 as follow 60° C for 5min, and 3°C/min until 260°C. Injector and flame-ionisation detector (FID) temperatures were maintained at 230°C. Identification of flavour components in the chromatogram was accomplished by comparing the retention times to those of pure standards obtained from a separate GC analysis. Quantification of flavour components was done by comparing the sample peak area to that of the pure component (known amount) peak area. Encapsulation efficiency was determined as the amount of flavour in the complex after processing as a percentage of the amount added into the mixture (Jeon 2003)

Results and discussion

Coacervates size distributions

Fig.1(a) shows the size distribution of coacervates containing quercetin and lactose as a function of emulsification speed. Mean diameters increase from 2µm to 20µm when the emulsification speed decreases from 12 to 6 (Chang 2006). Diameter distributions of particles collected at different stage of emulsification-coacervation process and containing quercetin and lactose are shown in Fig.1(b). The shifts of size distributions is clearly visible. Vigorous homogenization at speed 12 with Braun kitchen hand mixer led to small size droplets with a mean diameter of 1µm. The latter increases with gentle magnetic stirring up to 2µm. Resting system without stirring led to 3µm mean diameters after 1 h and finally to 35µm after 15h. This confirmed the higher diameters obtained by slowing down the stirring of emulsions and the narrower becoming of the size distribution. Shown in Fig.1(c) is the size distribution of coacervates with and without quercetin and lactose collected after 10 min of high homogenisation at speed 6, 20 min of agitation with magnetic stirrer both at 30°C and rest for 5 min at 4±1°C. Both curves have the same range of particles distribution. The majority

of droplets were in relatively larger mean diameter zone (20µm). Presence of the relatively smaller mean diameter zone (2µm) suggests the increase of diameters with slowing down of stirring.

a)

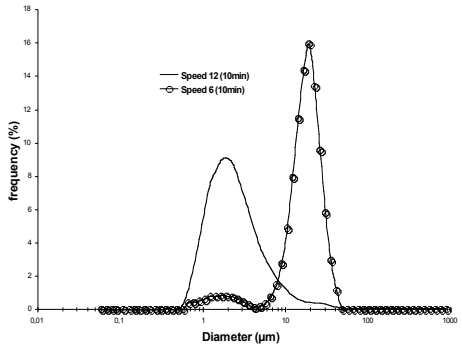

Fig. 1 a): Diameter distribution of the droplet with emulsification speed

b)

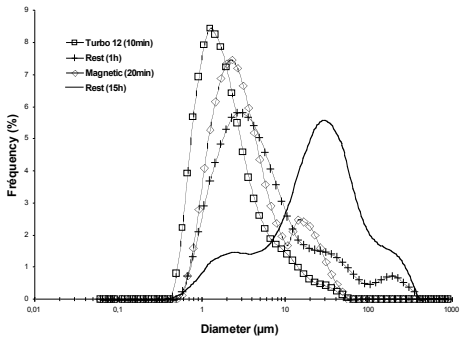

Fig. 1 b): Diameter distribution of the droplet at different stage of emulsification

Encapsulation efficiency

It is well known that Eucalyptus globules essential oil consists mainly of 1,8 cineol. The ones used in this study contained about 80% oil. Results of the total 1,8 cineol are presented in Table 1. It can be seen that encapsulation efficiency of coacervates with quercetin and lactose is higher than without those ingredients. Quercetin and lactose contribute to increase the amount of total 1,8 cineol entrapped.

Coacervates	with quercétine and lactose (%)	without quercétine nor lactose (%)
Encapsulation efficiency	50,08±14,63	18,97±5,5

Table1 : Encapsulation efficiency of 1.8 cinéol with composition of emulsion

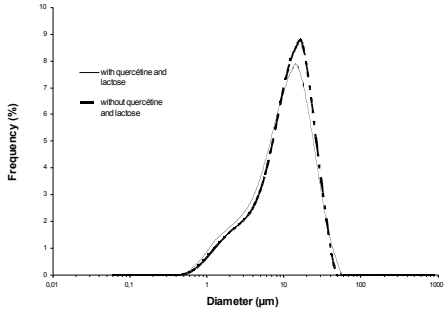

Fig. 1 c): Diameter distribution of the drople with emulsion composition

Microstructural properties of coacervates in emulsion and lyophilised coacervates

Optical contrast phase microscope and scanning electron microscope (SEM) were used to investigate respectively coacervates droplet in the emulsion; external–internal structures of encapsulated microcapsules prepared with and without quercetin and lactose using β-lactoglobulin and Acacia gum were observed for size and shape (Fig.2 and Fig.3). The Fig.2(a) show that coacervates were spherical. In that picture, (A) represents the transparent coacervates without oil and (B) the coacervates with oil. Fig.2(b) is the picture of coacervate phase after emulsification. The internal structures of coacervates without (resp with) quercetin and lactose are shown in Fig.3 A (resp. Fig.3 B). In both cases small holes were observed in the shell of wall matrices. Generally, small holes should contain droplets of flavors in the case of encapsulated liquid flavor (Shaikh, 2006). A1 exhibits plate forms while B1 present some spherical ones. A3 show compact form while in B3 there is a building shell that can content oil. Finally in A4 we can see the large and few holes from which oil can easily flow out while in B4 there are small holes making increasing the surface retention of the flavour compounds.

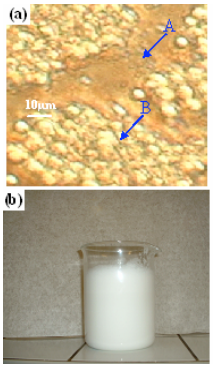

Fig. 2 : (a) Optical phase contrast microscopy of coacervate emulsion (b) picture of coacervate emulsion

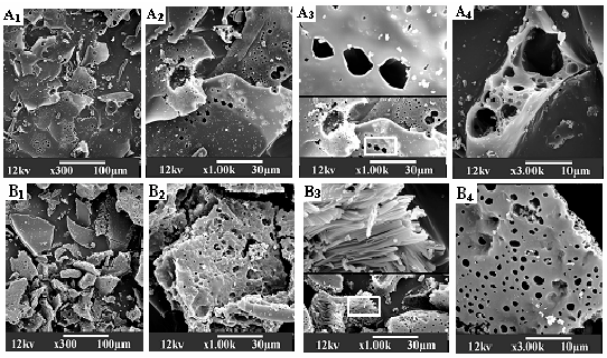

Fig. 3: Scanning electron micrographotograph prepare (A) without and (B) with quercetine and lactose

Conclusion

The β-lactoglobulin and Acacia gum system can be successfully used as wall materials to obtain lyophilised complex coacervates of Eucalyptus globulus essential oil. The use of lactose and quercetin largely increases the encapsulation efficiency and ameliorates the form of dried microcapsules. The results of this study draw hypothesis of the potential of quercetin as substitute of cancerous agent generally used to strengthen coacervate microcapsules as glutaraldehyde.

Acknowledgements

This study was partly supported by AUF (Agence Universitaire de la Francophonie)

Bibliography

A. Giamakis, O. Kretsi, I. Chinou, C. G. Spyropoulos (2001) Eucalyptus *camaldulensis: volatiles from immature flowers and high production of 1,8-cineole and β-pinene by in vitro cultures* Phytochemistry **58**, 351–355

C. G.deKruif, F. Weinbreck, R. de Vries (2004) Complex *coacervation of proteins and anionic polysaccharide* Current Opinion in Colloid & Interface Science **9**, 340–349

C-P. Chang, T-K. Leung, S-M. Lin, C-C. Hsu (2006) *Release properties on gelatin-gum arabic microcapsules containing camphor oil with added polystyrene* Colloids and Surfaces B: Biointerfaces **50** 136–140

C. Sanchez and D. Renard (2002) *Stability and structure of protein–polysaccharide coacervates in the presence of protein aggregates* International Journal of Pharmaceutics **242**, 319–324

D. Renard *,P. Robert, L. Lavenant, D. Melcion, Y. Popineau, J.Gue´guen, C. Duclairoir, E. Nakache, C. Sanchez, C. Schmitt (2002) *Biopolymeric colloidal carriers for encapsulation or controlled release applications* International Journal of Pharmaceutics **242**, 163–166

J. Shaikh, R. Bhosale, R. Singhal (2006) *Microencapsulation of black pepper oleoresin* Food Chemistry 94 105–110

R. Baranauskiene, P. R. Venskutonis, K. Dewettinck, R. Verhé (2006) *Properties of oregano (Origanum vulgare L.), citronella (Cymbopogon nardus G.) and marjoram (Majorana hortensis L.) flavors encapsulated into milk protein-based matrices* Food Research International **39** 413–425

Y-J. Jeon, T. Vasanthan, F. Temelli, B-K. Song (2003) *The suitability of barley and corn starches in their native and chemically modified forms for volatile meat flavor encapsulation* Food Research International **36** 349–355