XXV Encontro Nacional

Sociedade Portuguesa de Química

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Microencapsulation of *Ammodaucus leucotrichus* essential oil using chitosan/ TPP/vanillin chemical system

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A. leucotrichus (Coss. & Dur.) Coss. & Dur., known in Algeria as "Kammûnes-sofi", is a medicinal plant that finds culinary use by indigenous populations. Among others, it is used against stomach pain, indigestion, diarrhea, vomiting, fever, and to combat high blood pressure. In this work, the essential oil of *A. leucotrichus*, obtained by steam distillation (3h) from fruits collected in March 2015 from Tassili n'Ajjer, a vast plateau in south-east Algeria (25°30'0" N and 9°0'0" E), was chemically and biologically characterized and thereafter microencapsulated using a chitosan/TPP/vanillin system. Chemical characterization allowed the identification of ten constituents representing 98.6% of the whole essential oil composition. Oxygen-containing monoterpenes (87.2 %) were found to be the main group of components, followed by monoterpene hydrocarbons (11.1 %) and oxygen-containing sesquiterpenes (0.35 %). Perilla aldehyde was identified as the main component present in the essential oil accounting for 85.6 % of the total composition. Additionally, the oil presented antioxidant (EC_{50} 28±2 mg/ml, concentration able to scavenge 50% of DPPH radicals), anti-inflammatory (EC_{50} 11.7±0.7 µg/ml, concentration able to inhibit 50% of NO formation) and antimicrobial (against *Escherichia coli*: minimum inhibitory concentration (MIC) 10 mg/ml and minimum bactericidal concentration (MBC) 10 mg/ml; against *Staphylococcus aureus:* MIC 20 mg/ml and MBC 20 mg/ml) activities.*A. leucotrichus* essential oil microparticles were produced using an atomization/coagulation technique with chitosan as the shell material, sodium tripolyphosphaste (TPP) and vanillin as crosslinking agents. Comparatively to the most used chemical systems, this one presents several advantages since all the raw materials are nontoxic and no organic solvents are required. Moreover, the used microencapsulation process allows the microparticles production in a single step, without having the constrains of the traditionally used oil-in-water (o/w) emulsion based techniques. The adopted procedure comprises the following stages: (1) Chitosan solution (CS) preparation (3.0%, w/v) in acidic medium (acetic acid 3%, v/v); (2) Oil-in-water (o/w) emulsion preparation by emulsifying the essential oil (O) with the chitosan solution at O/CS ratio of 0.025 (v/v) with Tween 80 (emulsifier of HLB=15.0, 1.5%, w/v). The emulsion was homogenized at 11000 rpm during 5 min with a CAT Unidrive X homogenizer; (3) Atomization of the o/w emulsion in a Nisco VarJ30 system (flow rate: 0.3 ml/min) under pressurized nitrogen; (4) Coagulation with TPP (10%, w/v at pH 6.0) followed by vanillin crosslinking (1.0% (w/v), 50ºC at 0.5 ml/min during 2 h). Microparticles were recovered by filtration under reduced pressure, washed with distilled water and stored in the hydrated form.The produced microparticles were preliminary analyzed by optical microscopy (OM) using a Nikon eclipse 50i microscope to access size and morphology. This analysis showed the presence of spherical and individualized structures with an estimated particle size between 15 and 75 µm. Moreover, microparticles chemical structure was analyzed by FTIR, the thermal degradation was evaluated by TG and microparticle size distributions were measured by laser diffraction. The results shown the production of viable microparticles, indicating that the chitosan/ TPP/vanillin chemical system is a feasible alternative for a green *A. leucotrichus* essential oil encapsulation, when the atomization/coagulation technique is used. Moreover, taking into account the antimicrobial activity of *A. leucotrichus* essential oil, the produced microparticles can be a good alternative for cosmetic application as preservative.

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LSRE-LCM SHAKING THE PRESENT SHAPING THE FUTURE

Microencapsulation of *Ammodaucus leucotrichus* essential oil using chitosan/ TPP/vanillin chemical system

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INTRODUCTION

A. leucotrichus Coss. & Dur., known in Algeria as "Kammûnes-sofi", is a medicinal plant that finds culinary use by indigenous populations. Among others, it is used against stomach pain, indigestion, diarrhea, vomiting, fever, and to combat high blood pressure. In this work, the essential oil of *A. leucotrichus*, obtained by steam distillation (3h) from fruits collected in March 2015 from Tassili n'Ajjer, a vast plateau in south-east Algeria (25°30'0" N and 9°0'0" E), was chemically and biologically characterized and thereafter microencapsulated using a chitosan/TPP/vanillin system.

EXPERIMENTAL METHODS

***A. leucotrichus* plant with fruits.**

A. leucotrichus essential oil (ALOE) was characterized in terms of chemical composition (GC-FID and GC-MS) and bioactive properties: (i) antioxidant activity using DPPH and Reducing Power methods; antimicrobial activity by microdilution method; and anti-inflammatory activity by NO inhibition assay;

Microencapsulation of *A. leucotrichus* essential oil

Microencapsulation of ALOE was performed using an atomization/coagulation technique using a Nisco VarJ30 system, with chitosan as shell material, sodium tripolyphosphate (TPP) and vanillin as crosslinking agents. Fig. 1 presents the encapsulation system and a schematic representation of the ALOE encapsulation process.

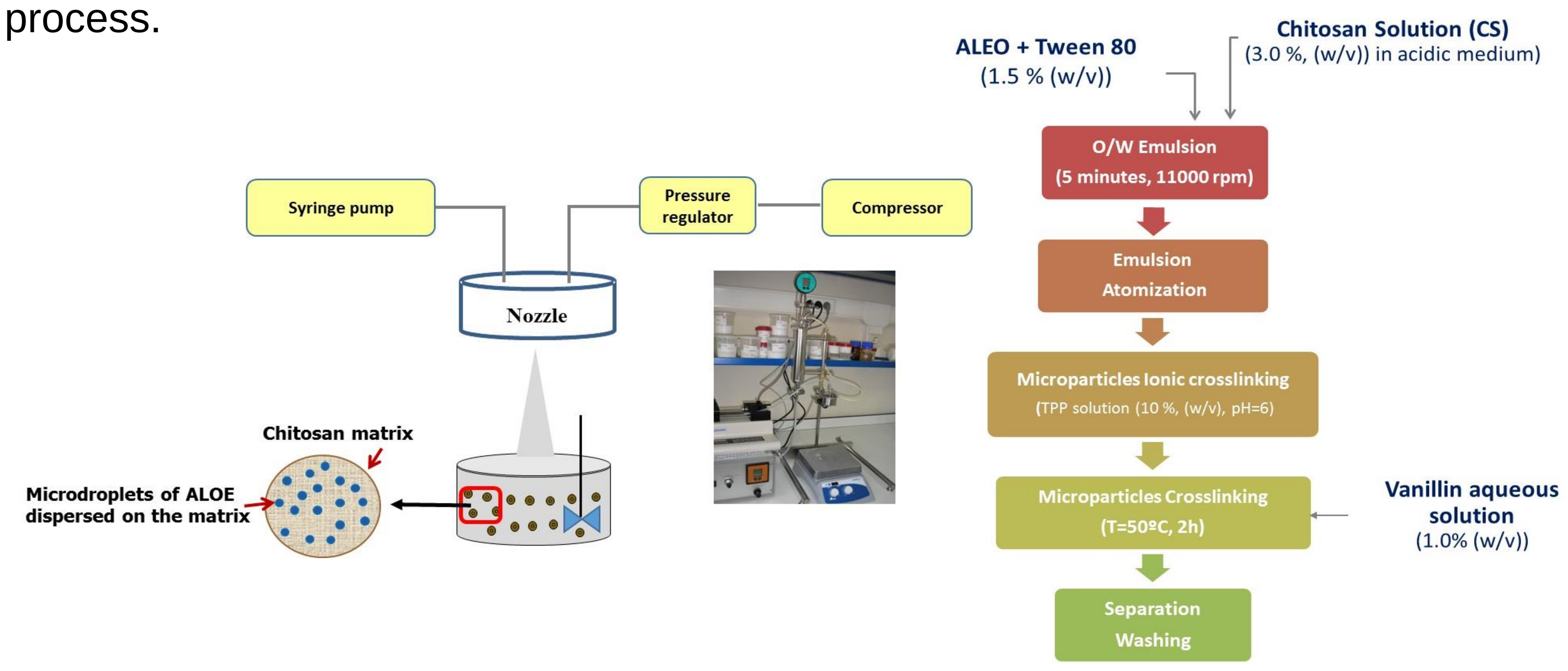


Fig. 1 Microencapsulation system Nisco Var J30 and schematic representation of the ALOE encapsulation process, where the different stages are identified.

Microparticles characterization

Preliminary analysis by optical microscopy (OM) using a Nikon eclipse 50i microscope was performed to access size and morphology;

Chemical structure analysis by FTIR, using an ABB equipment (model MB3000) in transmittance mode, by preparing KBr pellets with a sample concentration of 1% (w/w). Spectra were recorded between 500 and 4000 cm^{-1} at a resolution of 10 cm^{-1} and co-adding 32 scans. The following samples were analyzed: chitosan (Ch), microparticles of chitosan and TPP (MCS(Ch-TPP), microparticles of chitosan TPP containing ALOE (MCS(Ch-TPP-ALOE) and microparticles of chitosan, TPP and vanillin containing ALOE (MCS(Ch-TPP-Van-ALOE);

Thermal degradation evaluation by TG, using a Netzsch TG (model 209 F3 Tarsus), working in a temperature range of 30 to 600°C under inert atmosphere. All the individual components of the chemical system and the produced microparticles were analyzed.

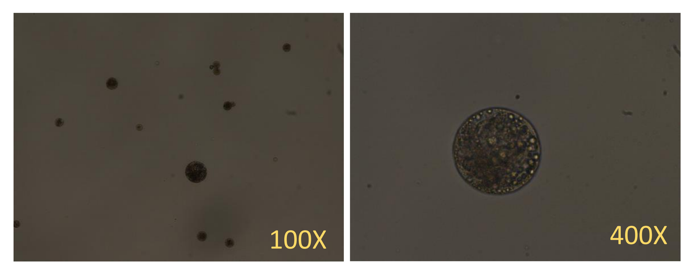
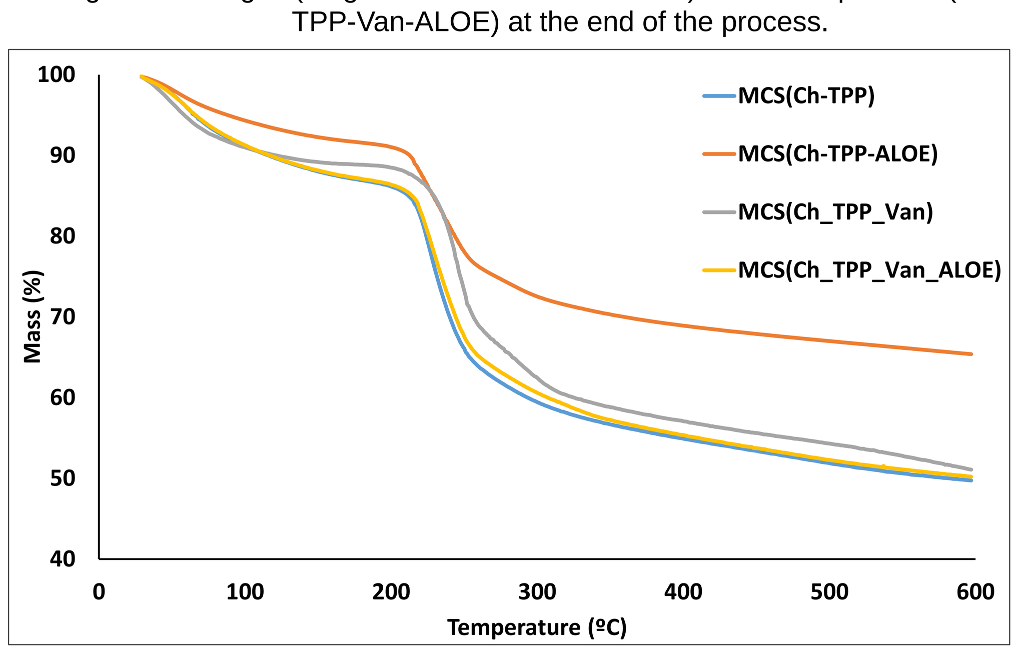
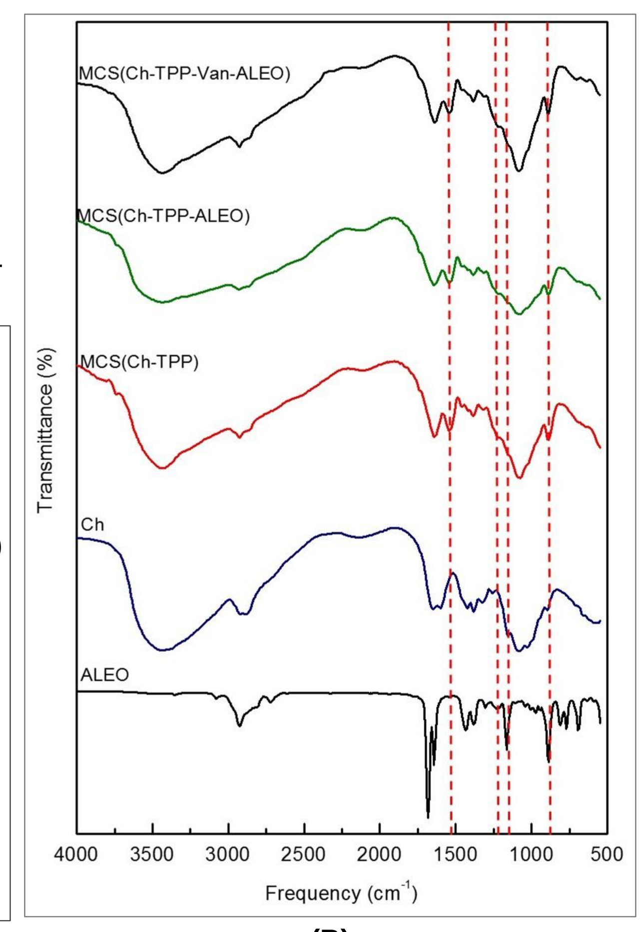


Fig. 2 MO images (magnification of 100 and 400X) of the sample MCS(Ch-TPP-Van-ALOE) at the end of the process.



(A)



(B)

Fig. 3 (A) Thermograms of the samples MCS(Ch-TPP), MCS(Ch-TPP-ALOE), MCS(Ch-TPP-Van) and MCS(Ch-TPP-Van-ALOE), and (B) FTIR spectra of ALOE, Ch, MSC(Ch-TPP), MCS(Ch-TPP-ALEO) and MCS(Ch-TPP-Van-ALOE).

RESULTS AND DISCUSSION

Table 1 Chemical composition of ALOE.

	Components	GC-FID		GC-MS		%
		LRI[a]	LRI[b]	LRI[a]	LRI[b]	
1	α-Pinene	1011	1008	928	939	2.2±0.1
2	Camphene	1047	1050	943	954	0.20±0.01
3	β-Pinene	1101	1113	971	979	0.37±0.02
4	3-Carene	1144	1147	1005	1011	0.36±0.02
5	Myrcene	1161	1160	986	989	0.11±0.03
6	Limonene	1176	1198	1023	1029	7.8±0.4
7	Perilla aldehyde	1738	1793	1289	1272	85.6±0.4
8	Methyl perillate	1831	-	1488	1394	0.92±0.02
9	Perilla alcohol	1966	2002	1297	1295	0.67±0.01
10	Spathulenol	2025	2085	1591	1578	0.35±0.03
	% Identified compounds					98.6
	Monoterpene hydrocarbons					11.1
	Oxygen-containing monoterpenes					87.2
	Sesquiterpene hydrocarbons					-
	Oxygen-containing sesquiterpenes					0.35

Table 2 Bioactive properties of ALOE.

Bioactive properties	ALOE
Antioxidant activity (EC_{50} values, µg/mL)	
DPPH scavenging activity	27.82±1.81
Reducing power	4.55±0.08
Anti-inflammatory activity (EC_{50}, µg/mL)	11.70±0.73
Antimicrobial activity (mg/ml)	
Escherichia coli (MIC)[1]	10.0
Escherichia coli (MBC)[2]	10.0
Staphylococcus aureus (MIC)	20.0
Staphylococcus aureus (MBC)	20.0

[1] Minimum inhibitory concentration; [2] Minimum bactericidal concentration.

Based on the results described in Table 1, the ALOE chemical characterization allowed the identification of ten constituents (98.6% of ALOE composition):

- Oxygen-containing monoterpenes (87.2%) as the main group of components,
- Monoterpene hydrocarbons (11.1%); oxygen-containing sesquiterpenes (0.35%).
- Perilla aldehyde was identified as the main component present in the ALOE (85.6% of the total composition).

According to the results in Table 2, ALOE presents antioxidant, anti-inflammatory and antimicrobial (against Gram negative bacteria *Escherichia coli* and Gram positive bacteria *Staphylococcus aureus*) activities.

The microparticles MO analyses showed the presence of spherical and individualized structures, as it can be seen in Fig. 2, with an estimated particle size between 15 and 75 µm. Moreover, no oil loss was observed.

TGA results shown in Fig. 3 (A), pointed out for the following conclusions:

- Ionic crosslinking of chitosan with TPP decreased the maximum degradation temperature of the resulting polymeric structure (from 298ºC of pure Ch to 227ºC with TPP addition), due to the reduction of chitosan crystallinity by phosphate groups incorporation.
- When ALOE is encapsulated with chitosan and TPP, (MCS(Ch-TPP-ALOE) a new degradation zone was identified for higher temperatures, being accompanied by an increased of the final residue to 65.4% (comparatively with MCS(Ch-TPP)) residue of 49.7%). This was probably due to a chemical interaction between chitosan and the perilla aldehyde of ALOE, resulting in a Schiff base generation;
- When the crosslinker vanillin was added, the interaction identified between chitosan and ALOE is decreased.

The FTIR analysis (Fig. 3 (B)) confirmed the reaction between chitosan $NH3^{+}$ and TPP phosphate groups, since the chitosan peak at 1599 cm^{-1} moved to lower frequencies (1542 cm^{-1}). This is further associated with a new peak identified at 1212 cm^{-1} and with the peak increasing at 1157 cm^{-1}, due to the P=O group presence. Moreover, the Schiff base resulting from the reaction of the vanillin aldehyde group with the $NH3^{+}$ of chitosan was confirmed by the peak increasing at 1637 cm^{-1}, being assigned to the generated C=N group.

CONCLUSIONS

The results shown the production of viable microparticles, indicating that the chitosan/ TPP/vanillin chemical system is a feasible alternative for a green ALOE encapsulation, when the atomization/coagulation technique is used. Nevertheless, further studies will be performed in order to minimize the chemical interaction between perilla aldehyde from ALOE and chitosan. Moreover, taking into account the antimicrobial activity of ALOE, the produced microparticles can be a good alternative for cosmetic application as preservative.

ACKNOWLEDGEMENTS

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