

New Technologies in Rice Derivatives

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Summary In this research the encapsulation of ferulic acid (FA) in advanced systems of protection with the aim of improving its stability and photostability was studied. Lipoparticles and polymeric microparticles as encapsulation systems were prepared and characterized. Lipoparticles were completely of natural origin, while microparticles were obtained using chitosan as natural polymer. In both systems FA stability was greatly increased and its organoleptic properties in the emulsions did not change.

Key Words ferulic acid, lipoparticles, polymeric microparticles, stability, photostability

Ferulic acid (FA) is well known for its properties as multiactive ingredient. Indeed it can be used, as examples, for its photoprotective and antiradical action or antimicrobial property. FA can be found in rice, in wheat, in barley, and in other natural products (1, 2). Above all it is one of the main constituents of rice and as such today it is increasingly appreciated for its properties. In fact the actual trend in food and cosmetic fields gives increasing importance to the use of natural substances. Recently a special attention is dedicated to the use of environmentally friendly substances in view of bio- and environmental soundness. In Japan, the use of FA as sunscreen is allowed: this aspect must be kept in great consideration since, in these last years, researches are showing more and more clearly the role of physical and chemical sunscreens in seas pollution above all in coastal areas and in coral reef (3, 4). However, the use of FA in formulations has a drawback: it is subject to degradation with time that in turn brings about a variation in its organoleptic properties (5). In order to stabilize FA, it has been inserted in various kind of systems as: supramolecular systems that use cyclodextrins (6, 7), liposomes (8), and SLN (nanoparticles) (9, 10) but there are no references on the use of natural lipoparticles containing FA. With regard to the use of chitosan microparticles there are two references about the preparation of chitosan nanoparticles and matrixes containing FA with the aim of controlling the growth of microorganisms (*Candida Albicans* and *Aspergillus parasiticus*) (11, 12). In the literature are also cited microparticles containing FA obtained with the use of a synthetic polymer (Eudragit) in a spraydrying process (13). These microparticles are possible carriers, in “oral drug delivery”, for controlled release of FA. The research concerning the polymeric microparticles was oriented toward the building of these systems using a natural polymer (chitosan) to protect the molecule (FA) and toward the evaluation of the possible applications in the sun protection field.

With this purpose we have prepared lipoparticles and polymeric microparticles containing FA to evaluate the

efficacy and the safety of the formulations in which the particles have been encapsulated.

Materials and Methods

Lipoparticles have been prepared by Melt Dispersion Techniques which is mainly suited for lipophile active ingredients but it is also applied in the encapsulation of hydrophilic substances (14). This method does not use organic solvents thus avoiding the risk of possible poisonous effects (due to the presence of their residues). The process is based on the dispersion of the active ingredient in the melted lipids and successive emulsification of this mixture with an aqueous solution containing the surfactant.

In our case it was necessary to do some changes due to the poor solubility of FA in water and in lipids. Thus, the melted lipidic phase, containing the emulsifier and the active ingredient previously solubilized in the least quantity of ethanol, was emulsified in water. The oil/water (O/W) emulsion so obtained was then cooled in an ice bath with consequent formation of solid lipid particles. These last have been recovered by centrifugation and then dried in a vacuum dryer. Using the same process we also prepared the lipoparticles without the active ingredient in order to validate the obtained results.

The particles have been obtained using as solid lipid, a triglyceride (tripalmitin) and a non-ionic emulsifier of natural origin (Olivem 1000; Cetearyl Oliviate, Sorbitan Oliviate). Besides an other non-ionic emulsifier of natural origin has been used (Montanov 68, Cetearyl Alcohol, Cetearyl Glucoside) in order to evaluate its influence in the encapsulation of FA in the lipoparticles (Tables 1 and 2).

Lipoparticles obtained were examined by optical microscopy (OM) and scanning electron microscopy (SEM) to characterize their morphology, and ultraviolet (UV) spectrometry to assess the efficiency of encapsulation and the loading.

The stability and photostability of FA free and encapsulated in lipoparticles were evaluated in O/W emulsions. The emulsion composition is reported in the Table 3. The concentration of FA has been determined by high

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Table 1. Composition of formulations for the preparation of lipoparticles.

Ingredients	Formulations				
	1	2	3	4	5
Tripalmitin	1.40	1.40	1.40	1.40	1.80
Olivem 1000	0.23	0.23	0.23	0.23	0.29
Water	q.s.	q.s.	q.s.	q.s.	q.s.
	20.00	20.00	20.00	20.00	20.00
Ethanol	2.80	2.80	2.80	6.00	4.00
Ferulic acid	—	0.25	0.45	0.90	—

Table 2. Composition of formulations for the preparation of lipoparticles.

Ingredients	Formulations				
	6	7	8	9	10
Tripalmitin	1.80	1.80	2.00	1.80	1.80
Olivem 1000	0.29	0.29	0.33	—	—
Montanov 68	—	—	—	0.29	0.29
Water	q.s.	q.s.	q.s.	q.s.	q.s.
	20.00	20.00	20.00	20.00	20.00
Ethanol	4.00	5.00	4.00	4.00	4.00
Ferulic acid	0.60	0.80	0.60	—	0.60

Table 3. Composition of emulsion containing FA lipoparticles and microparticles.

Ingredients*	E1	E2	E3
Cetearyl Oliviate, Sorbitan Oliviate	5.00	5.00	5.00
Cetearyl Isononanoate	5.00	5.00	5.00
C12–15 Alkyl Benzoate	10.00	10.00	10.00
Dicaprylyl Ether	5.00	5.00	5.00
Methylparaben	0.10	0.10	0.10
Propylparaben	0.10	0.10	0.10
Xanthan Gum	0.30	0.30	0.30
Aqua	q.s.100	q.s.100	q.s.100
Disodium EDTA	0.15	0.15	0.15
Sodium Benzoate	0.30	0.30	0.30
Potassium Sorbate	0.30	0.30	0.30
Ferulic Acid	—	2.00	**
Triethanolamine	—	1.49	—

* Quantities are given in gram; ** encapsulated filter corresponding to 2% FA; E1: basic emulsion; E2: emulsion containing free FA; E3: emulsion containing encapsulated FA.

performance liquid chromatography (HPLC) in the stability test at different times.

In order to determine the photostability the emulsions have been irradiated using a solar simulator at 10 (300 mJ/cm²) and 20 (600 mJ/cm²) MED (minimal erythemal dose) and the percentage of FA evaluated by

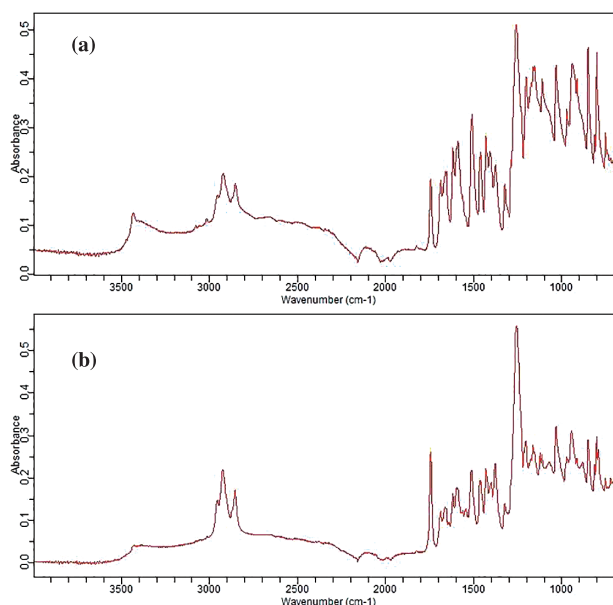


Fig. 1. FT-IR spectra: (a) FA-loaded microparticles; (b) physical mixture (empty microparticles+FA).

HPLC.

The SPF (Sun Protection Factor) *in vitro* has been also evaluated both before and after irradiation at 10 MED on all the emulsions.

Antiradical activity evaluation has been done on a solution of free FA in ethanol and on a dispersion of encapsulated FA in ethanol using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) test.

The redox behavior has been evaluated by cyclic voltammetry (15, 16).

Polymeric microparticles were prepared using chitosan (supplied by Sigma-Aldrich) a natural polymer endowed with biocompatibility, low toxicity, biodegradability and substantivity for keratin.

Microparticles have been prepared by ionic reticulation method using sodium triphosphate pentabasic (STP) or sodium hydroxide as crosslinking agents (17).

STP put in contact with chitosan forms a gel through ionic interactions (18). The addition of the chitosan solution has been done drop by drop with syringe or by nebulization with a spray distributor. Chitosan with low or medium molecular weight (MW) has been used.

Microparticles were characterized by morphological analysis using OM. Loading evaluation was done by spectrophotometric analysis. The encapsulation of FA was confirmed by Fourier transform infrared (FT-IR) spectroscopy. The analysis has been done on the empty particles, on free FA, on the encapsulated FA and on the physical mixture (empty particles+FA) (Fig. 1).

Microparticles obtained have been put in O/W emulsions (see Table 3) to evaluate stability and photostability.

Results

The encapsulation of ferulic acid in lipoparticles has shown interesting results. In the formulas reported in Tables 1 and 2 the ratios lipid/emulsifier were changed

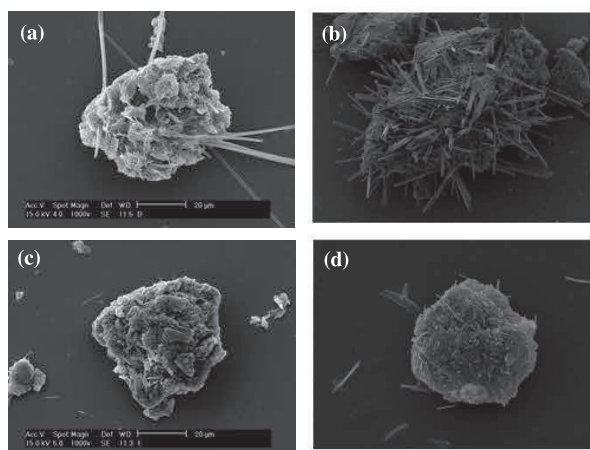


Fig. 2. SEM photographs of FA-loaded lipoparticles: (a) and (b) sample 6 not washed; (c) and (d) sample 6 washed with ethanol.

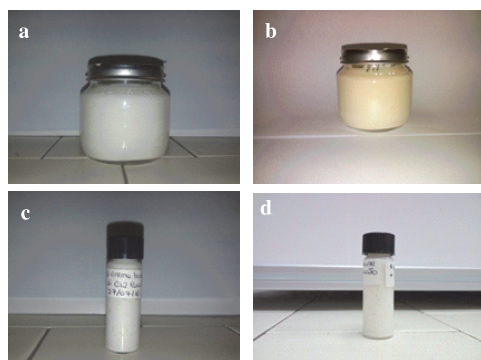


Fig. 3. Stability of emulsions containing free FA (a: T₀; b: T₁) and FA-loaded lipoparticles (c: T₀; d: T₁) after 1 mo.

with the aim of evaluating their influence on the loading of FA. Formulation 6 shows the best loading as we can see from loading determination done by UV analysis. The results of encapsulation efficiency are reported for emulsions containing washed (with ethanol) and not washed lipoparticles. The OM and SEM analyses have shown homogeneous particles but in not washed samples needles structures have been noted at the exterior of the particles (see Fig. 2).

Loading efficiency decreased with the increasing of the quantity of FA in the preparation. Regarding the applicative properties it was important to evaluate the stability of the emulsions with time and the photostability. In emulsions with free FA the organoleptic properties (colour) changed with time as can be seen in Fig. 3. There was no change of colour in emulsions containing washed lipoparticles of FA after one month. As can be seen from Table 4 the concentration of FA evaluated by HPLC at T₀ and T₁ (one month) decreased only by 0.5% while for free FA the degradation was 11%.

Regarding photostability, the degradation of FA in emulsions was 56% for free FA and 7.8% for encapsulated FA.

Table 4. Stability of ferulic acid loaded lipoparticles.

Emulsions	% FA		% degradation
	24 h	1 mo	
FA free	2.20±0.05	1.96±0.08	10.91
FA encapsulated	2.19±0.60	2.18±0.28	0.46

Table 5. SPF values evaluated before and after irradiation of FA free and encapsulated in lipoparticles.

Emulsions	SPF	
	Before irradiation	After irradiation
FA free	3.60±0.16	3.21±0.17
FA encapsulated	2.22±0.18	2.08±0.12

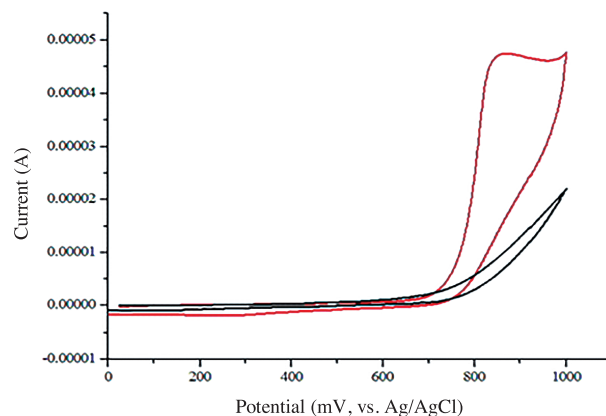


Fig. 4. Cyclic voltammograms obtained with the platinum electrode on the solution containing free FA (line red) and on the solution containing FA encapsulated (line black).

SPF in vitro evaluation is shown in Table 5.

Antiradical activity was reduced in FA encapsulated in comparison with free FA. (IC₅₀=23.773±4.393 μg/mL and IC₅₀=12.365±0.322 μg/mL for encapsulated and free FA respectively).

Redox behavior for lipoparticles containing FA, reported in Fig. 4 has shown an irreversible voltammogram while that of free FA was reversible. This fact shows that FA was not released in alcoholic solution by lipoparticles for which there was not an anodic peak potential. Thus there was not any oxidation.

The encapsulation of FA in microparticles gave homogeneous particles with varying dimensions as resulted from OM analysis. The dimensions of the particles obtained with different quantities of FA and low and medium MW chitosan are shown in Table 6. The evaluation of FA loading was done by spectrophotometric analysis on samples containing FA after solubilization in a tetrahydrofuran (THF)/H₂O (9:1) mixture

Table 6. Dimension of the microparticles obtained with low and medium MW chitosan.

Sample	Amount FA in emulsion	Size (μm)	MW/Amount (g) Chitosan
19	0.2	53.60 (± 14.48)	Medium/0.03
20	0.2	46.79 (± 6.71)	Low/0.12
21	0.2	46.23 (± 11.37)	Low/0.03
22	0.5	34.09 (± 15.93)	Medium/0.03
23	0.5	21.39 (± 2.18)	Low/0.12
24	0.5	26.79 (± 3.59)	Low/0.03
25	1.0	37.04 (± 1.00)	Medium/0.03
26	1.0	52.24 (± 11.07)	Low/0.12
27	1.0	37.17 (± 3.40)	Low/0.03

Table 7. Loading and Encapsulation Efficiency of polymeric microparticles.

Sample	Amount FA in emulsion	Loading (\pm SD)	Encapsulation Efficiency (%)
19	0.2	2.26 (± 0.29)	21.36
20	0.2	2.67 (± 1.29)	26.43
21	0.2	3.56 (± 1.28)	33.64
22	0.5	4.68 (± 1.39)	20.49
23	0.5	9.06 (± 1.28)	41.31
24	0.5	13.50 (± 1.27)	59.13
25	1.0	24.18 (± 2.74)	65.05
26	1.0	25.52 (± 1.57)	70.94
27	1.0	37.22 (± 6.83)	100.0

and the results are reported in Table 7. FA loading was further evaluated before and after washing with ethanol to eliminate FA not encapsulated (see Table 8). The encapsulation of the active ingredient (FA) in microparticles was evaluated by FT-IR analysis. The analysis has been done on the empty particles, on the free FA, on the encapsulated FA, and on the physical mixture (empty particles+FA). In Fig. 1, the corresponding spectra are shown. Microparticles obtained have been put in O/W emulsions. Their protective efficacy has been evaluated and compared with that of emulsions containing free FA. The composition of emulsions are reported in Table 3. The organoleptic characteristics of the emulsions have been evaluated; the emulsions containing encapsulated FA did not change their colour after one month at room temperature. The spectrophotometric analysis revealed a decrease in SPF that can be attributed to the screening action of the polymeric matrix.

Microparticles containing FA and emulsions containing free and encapsulated FA respectively have been irradiated at 10 MED. The results are reported in Table 9.

Discussion

From the results obtained it can be deduced that lipoparticles are an efficient encapsulating system to avoid the degradation of FA and thus they allow its use in

Table 8. Loading and Encapsulation Efficiency of polymeric microparticles after washing with ethanol.

Sample	Loading (%) BW	Loading (%) AW	Loading (%) Variation	EE (%)
25	24.18 (± 2.74)	23.16 (± 0.97)	4.22	62.30
26	25.52 (± 1.57)	24.50 (± 1.23)	3.99	68.11
27	37.22 (± 6.83)	35.05 (± 4.21)	5.83	94.29

BW: before washing; AW: after washing; EE: encapsulation efficiency.

Table 9. Photostability of ferulic acid loaded microparticles.

Sample	Loading% BI	Loading% AI 10 MED	Loading (%) Variation
27	37.22 (± 6.83)	34.75 (± 1.25)	6.63
27 washed with ethanol	35.05 (± 4.21)	32.63 (± 2.45)	6.90
Physical mixture	34.62 (± 0.14)	24.61 (± 0.14)	28.90

BI: before irradiation; AI: after irradiation.

formulates whose properties will not change with time. Indeed FA concentration in lipoparticles, evaluated by HPLC, changes only by 0.5% (see Table 3) even after irradiation with a solar simulator that in turn caused a high variation in concentration (56%) in cases of use of free FA.

So the matrix protects the molecule, increasing its stability to irradiation, but it also lowers its SPF value (shielding effect due to the matrix). FA encapsulated in lipoparticles submitted to DPPH test (subject to steric hindrance) showed a decrease in anti-radical activity but it did not present oxidation (see Fig. 4).

We set a suitable method for the preparation of homogeneous microparticles containing FA. The FA loading was optimized modulating the MW of the chitosan and the FA percentage as a function of the required size of the particles (25–40 μm) (see Table 6). The best results in encapsulation efficiency have been obtained using low MW chitosan at lower concentration of chitosan (Table 7). The loading efficiency was poorly affected by washing with ethanol.

The FT-IR spectra showed that the encapsulation is effective: indeed the spectrum of the encapsulated system is different from that of the physical mixture (FA+empty particles) (Fig. 1).

As we observed for FA lipoparticles, also in the O/W emulsions, containing FA natural polymeric microparticles there was any change in organoleptic properties. This fact is a consequence of the stabilization and photostabilization of FA in these systems.

In conclusion this research highlighted the possibility of applying new technologies to rice derivatives. Green systems can be obtained in order to support eco-sus-

tainability. The products obtained, included in cosmetic formulations, have shown compatibility with the ingredients commonly used in cosmetics in both stability and photostability tests.

Disclosure of State of COI

The authors declare that there is no conflict of interest. Funds for researches were not provided by a company or a for-profit organization.

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