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Casein hydrolysate encapsulated in lyophilized liposomes

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INTRODUCTION AND OBJECTIVES

Liposomes are colloidal systems formed by aggregates originated from aqueous dispersions of phospholipids, and their structural characteristics allow them to encapsulate or incorporate hydrophilic, hydrophobic or amphiphilic substances. There are many potential applications for them in food; one of the most interesting is the encapsulation of functional bioactives, like protein hydrolysates and antioxidants (Mozafari et al., 2008). Alternative food formulations can be produced using liposomes; they can be an alternative, for example, to overcome the protein malabsorption or digestion problems related to the incomplete digestion of these biomolecules. Liposomal systems can highly improve their intestinal absorption (Jeong et al 2007), as well as hide the bitterness these hydrolysates often present (Morais et al 2003).
This work aimed to encapsulate casein hydrolysate (CH) in liposomes and submit these vesicles to a lyophilization step, characterizing the resulting systems. Two soy lecithins were tested, a hydrogenated and purified one (Lipoid S100-H, Lipoid, at least 97% phosphatidylcholine) and a non-purified and non-hydrogenated one (Lipoid S-40, Lipoid, a mixture of at least 40% phosphatidylcholine with 12-15% of phosphatidylethalonamime, and a minor percentage of phosphatidylinositol). The addition of two disaccharides (trehalose and sucrose) as cryoprotectors was evaluated by scanning electron micrography and X-ray diffraction. The hygroscopicity of the resulting powders was also measured.

MATERIALS AND METHODS

Liposome preparation The multilamellar liposomes, composed of purified and hydrogenated soy lecithin or soy lecithin partially purified (Lipoid S100-H and Lipoid S-40, respectively, both from Lipoid GmbH, Germany) were prepared by the hydration film method (final phospholipid concentration 7,5 mg/mL in all formulations). CH (Hyprol 8052, donated by Kerry Bioscience) and the cryprotectors (sucrose or trehalose, mass ratio 4:1 cryoprotector:phospholipid, based on Cabral et al 2004) were added in the hydration solution. Non-encapsulated CH was separated by ultrafiltration (membrane cut diameter 100 kDa). Lyophilization was carried out after freezing liposomes with liquid nitrogen. The formulations tested are explicited in Table 1.

Liposome characterization The liposomes were characterized by scanning electron microscopy, X-ray diffraction and powder higroscopicity.

Table 1: Liposome formulations used for the encapsulation of CH.

Formulation	Lecithin	Cryoprotector
S100-T	Purified (n.l.t. 97% choline) and hydrogenated	Trehalose
S100-S		Sucrose
S40-T	n.l.t. 40% choline, non-hydrogenated	Trehalose
S40-S		Sucrose

RESULTS AND DISCUSSION

The encapsulation efficiencies of all analysed systems were between 38 and 50%. Scanning electron micrographies of the lyophilized systems are shown in Figure 1:

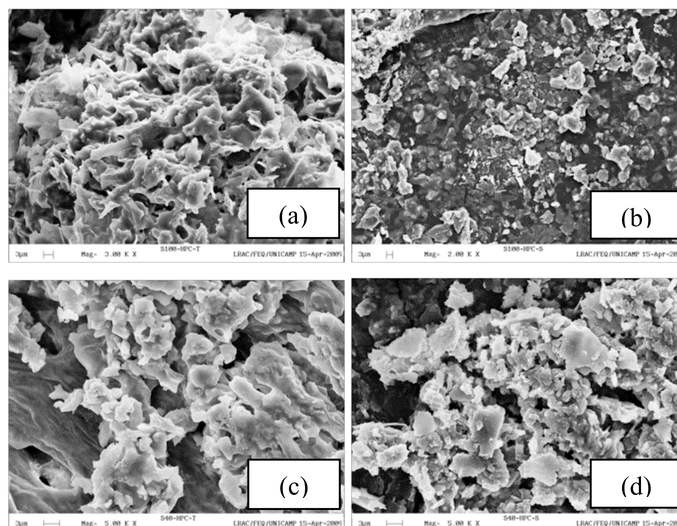

Figure 1: Scanning electron micrographies of lyohilized liposomes: formulations (a) S100-T (magnification 3000x); (b) S100-S (magnification 2000x) (c)S40-T (magnification 5000x); (d) S40-S (magnification 2000x).

In all cases it is possible to distinguish the lyophilized liposomes immersed in a matrix probably formed by excess cryoprotector with a visible amorphous characteristic, which is an indication that this structure is highly viscous and has low molecular mobility, two essential characteristics to protect the liposomes during the lyophilization pross (Wolkers et al. 2004). The lyophilized phospholipid vesicles are clear visible in the micrographies, and there are not major indications of rupture or morphological alterations.
Hygroscopicity assays, carried out according to protocol from Cai et al 2000 (using a relative humidity of 81%), resulted in the water absorption profiles as shown in Figure 2.

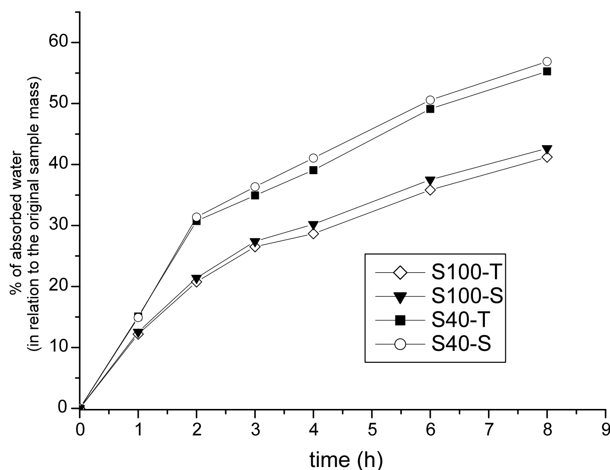

Figure 2: Higroscopicity profiles (81% relative humidity) of lyophilized liposomes encapsulating casein hydrolysate.

It is possible to conclude all tested samples presented high level of hygroscopicity, a result already expected, as the casein hydrolysate used (Hyprol 8052) is highly higroscopic. However, it can be noticed lyophilized S40 liposomes were more susceptible to water absorption than the correspondent S100 samples.

X-ray diffraction analyses had their results explicited in Figures 3 and 4:

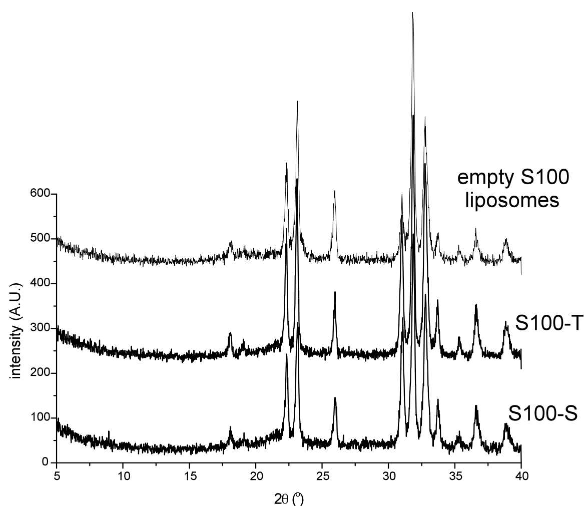

Figure 3: X-ray diffractograms of lyophilized liposomes produced with purified and hydrogenated phospholipid (Lipoid S100-H).

In both cases the lyophilized liposomes had X-ray diffraction profiles typical of amorphous materials (maximum peak intensities were 600 and 1000 for S100 and S40 liposomes, respectively). This is a very important characteristic, as the more amorphous the material, easier is to rehydrated it.

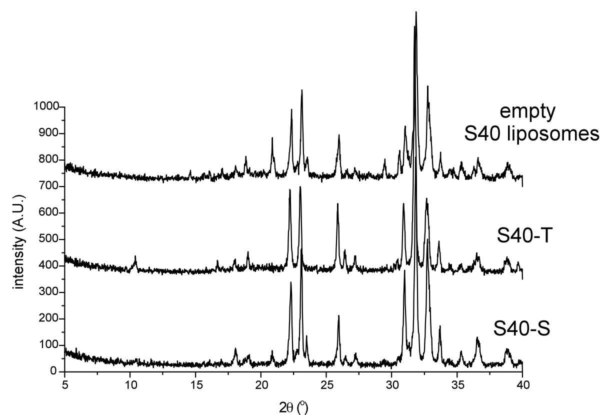

Figure 4: X-ray diffractograms of lyophilized liposomes produced with non-purified and non-hydrogenated phospholipid (Lipoid S40).

Another relevant characteristic that must be pointed out in these diffractograms is that they are not a simple superposition of HC, phospholipids and cryoprotectors diffraction patterns, but a completely different diffraction pattern, proving new structures were really formed.

CONCLUSIONS

The results indicated the feasibility of producing multilamellar liposomes encapsulating casein hydrolysate, in the lab scale, with good encapsulation efficiency, and afterwards submit them to a lyophilization step. These liposomes were produced using two different types of soy lecithin, and in both cases the incorporation of disaccharides as cryoprotectors was possible and they apparently protect the morphology of the phospholipid vesicles. The hygroscopicity of the samples was high in all cases, indicating special care must be taken in the storage of the samples.

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