P-007	**Buriti (*Mauritia flexuosa*) oil incorporation in lyophilized liposomes**	

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INTRODUCTION AND OBJECTIVES

Carotenoids are nowadays one of the most interesting ingredients in food formulations due to their antioxidant characteristics, which are able to confer functional properties. However, their bioavailability may be very low, since they are hydrophobic and their absorption depends not only on the release from the food matrix, but also on the subsequent solubilization by bile acids and digestive enzymes, resulting in their incorporation into micelles (Parada et al. 2007). From this point of view, lipid-based micro and nanostructures are very interesting to be applied as incorporation media for the carotenoids, but they have their exploitation in food technology yet to be explored (Mozafari et al. 2006).
The study here presented aimed to verify the feasibility of incorporating Buriti (*Mauritia flexuosa*) oil, a raw material very rich in carotenoids, extracted from an Amazon palm tree, in multilamellar liposomes, as well as the possibility of incorporation of cryoprotectors for the posterior lyophilization of these colloidal structures. The general purpose was to link the need of developing new alternatives to disperse/incorporate the hydrophobic carotenoids in food formulations to the use of a very carotenoid-rich and yet unexplored raw material as Buriti oil. Two cryoprotectors were tested in the lyophilization process, trehalose and sucrose.

MATERIALS AND METHODS

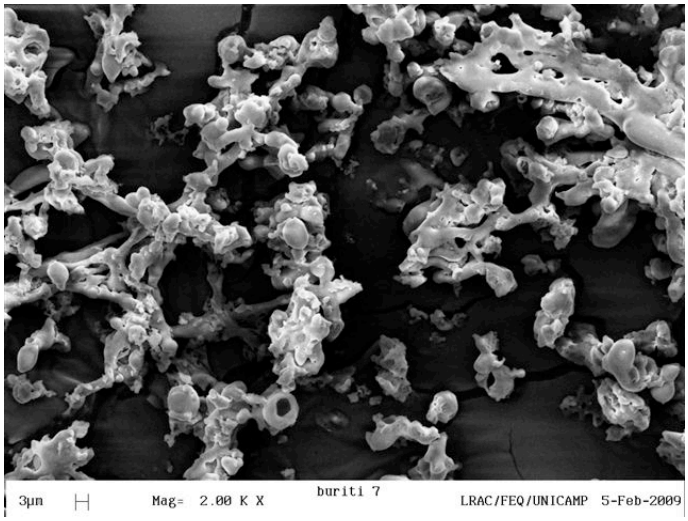
Liposome preparation The liposomes were composed of hydrogenated soy lecithin (Lipoid S100-3, Lipoid, Germany) and prepared by the hydration film method (final phospholipid concentration 6 mg/mL). The refined Buriti oil (BO) was a donation of Croda. Cryoprotectors tested were sucrose and trehalose.

Liposome rupture and quantification of total carotenoids The liposomes were ruptured and the carotenoids extracted using a liquid-liquid extraction with ethanol and hexane, followed by spectrophotometric analyses to determine the carotenoids concentration in the hexane phase.

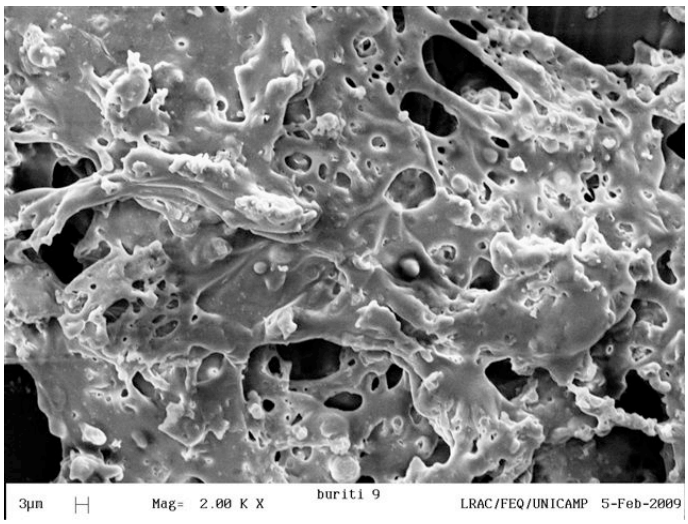
Characterization of the liposomes The liposomes were characterized by differential scanning calorimetry (DSC), scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and hygroscopicity.

RESULTS AND DISCUSSION

DSC analyses of the liposome aqueous dispersions originated thermogramas with a wide peak, but no phase separation occurred. This behavior reflects the heterogeneity of the bilayer composition, and the detection of just one peak, despite of its width, means this heterogeneity did not imply in phase separation.
Figure 1 shows the scanning electron micrographies of the powders resulting from the lyophilization of the liposomes with the two different cryoprotectors:



(A)



(B)

Figure 1: SEM of lyophilized liposomes: (A) formulation with trehalose (B) formulation with sucrose.

In the micrographies is possible to distinguish the lyophilized liposomes incorporated in the amorphous mass of excess cryoprotector, a configuration agreeing with the one described in the literature as the suitable to protect the phospholipid vesicles from the loss of the structural characteristics due to their fusion (Mobley et al.1994).

The carbohydrate in excess has a visible amorphous characteristic, *i.e*, a high viscosity and low molecular mobility, protecting the liposomes during the drying process and avoiding the approximation of the vesicles, and thus decreasing the probability of fusion. Also, the cryoprotection seemed to be very efficient in preserving the original morphology and diameters estimated for the vesicles in the aqueous dispersions.

The hygroscopicity of the lyophilized samples was tested in 81% RH environment (according to a protocol adapted from Cai et al. 2000), and the resulting water absorption curves are shown in Figure 2.

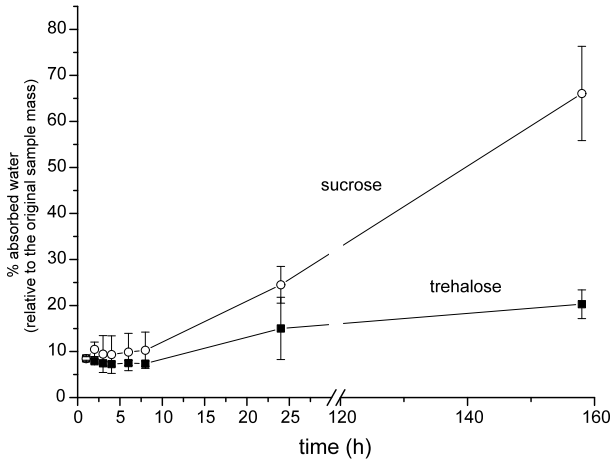


Figure 2: Hygroscopicity profiles of the lyophilized BO-loaded liposomes (81% RH).

The data indicate trehalose as a very much efficient protector against water absorption by the lyophilized sample in comparison to sucrose.

FTIR spectra showed the general aspect of both spectra for formulations with cryoprotectors were exactly the same, indicating the vibrational configurations of both systems are similar. Also, the vibration band located between 3600 and 3000 cm^{-1}, related to O-H stretching vibration, is much more pronounced in the lyophilized liposomes than in pure disaccharides, indicating the O-H groups of trehalose and sucrose effectively created hydrogen bonds in the system, revealing effective interaction among the O-H groups from the disaccharides and the polar groups of the phospholipid, as expected.

There is also a sharp decrease of the peak absorbance in the vibrational regions related to the microenvironment of the polar head of phospholipids, namely the stretching of P=O bond of phosphate group (peak at 1236 cm^{-1}) and C=O of esters (peak at 1736 cm^{-1}). Both decreases occur due to the interactions (hydrogen bonds) of cryoprotector O-H groups with polar head of phospholipids in the liposome polar region of the liposome membrane, restricting their motions.

Another absorbance decrease occurs at 1468 cm^{-1}. This is a characteristic band of rotationally disordered polymethylene chains (group $-(CH_2)_n-$), and this decrease suggests the packing of the lipid bilayer was altered when the cryoprotector was added, restricting the motion of these chemical groups, which apparently had their bending vibration highly limited.

The carotene typical strong bands between 990 and 960 cm^{-1}, due to CH out-of-plane deformation occurring in the polyene structure of the carotenoids, are not present in any of the formulations. This is typical of Buriti oil, in whose infrared spectrum there are not contributions of tocopherol or carotenoid typical groups. On the other hand, a strong band typical of unsaturated fatty acids, related to CH out-of-plane wagging of ethylene group, can be identified at 986 cm^{-1} in formulations 2 and 3.

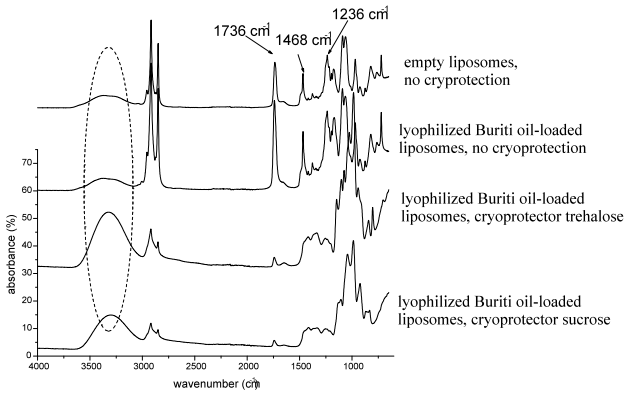


Figure 3: FTIR spectra of lyophilized liposomes.

CONCLUSIONS

It was possible to produce multilamellar liposomes incorporating Buriti (*Mauritia flexuosa*) oil, with no phase separation in the phospholipid membrane, even when cryoprotectors were added in the formulation. The lyophilization did not seem to destroy the liposomal structure, as showed by the scanning electron micrographies, and did not affect the interactions among the different components of the formulations. Trehalose was very efficient to protect the lyophilized liposomes against water absorption, much more than sucrose, although both disaccharides interact with the polar heads of phospholipid as evidenced by FTIR spectra.

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