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Development of nanoemulsions with tucumã (astrocaryum vulgare) fruits oil

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Abstract

Astrocaryum aculeatum is a palm species which belongs to the family Arecaeae, being commonly known as tucumã and widely distributed through Amazon. Tucumã fruit is a typically Amazonian raw material, which can be used for extraction of oil with great economic importance. However, to our knowledge, tucumã remain unexplored concerning development of a nanobiotechnology product. The aim of the present study was to develop nanoemulsions containing tucumã fruits oil. Formulations prepared with different blends of sorbitan monooleate and polysorbate 80 at HLB 11.0, 12.0, 12.25, 12.5, 12.75, 13.0, 13.25, 13.5, 13.75 and 14 were considered nanoemulsions. Smallest mean droplet (156.6±0.6557 nm) was observed for nanoemulsion at HLB 13, which was considered required HLB value of this oil. On this context, this study provides valuable information concerning nanobiotechnology of Amazonian oils with great interest for pharmaceutical, cosmetics and food industries.

Keywords: HLB, Nanoemulsion, Tucumã

Introduction

Astrocaryum aculeatum is a palm species which belongs to the family Arecaeae, being commonly known as tucumã and distributed through North (Acre, Amazonas, Pará, Rondônia, Roraima) and Central-West (Mato-Grosso) regions of Brazil.[1-3] It is also found through other countries, such as Bolivia, Colombia, French Guiana, Suriname, Venezuela and Trinidad and Tobago[4] Several biological activities, including anti-inflammatory.[5,6] antibacterial and antifungal.[7] properties have been reported for this species. Its fruits are important sources of minerals, including potassium, calcium, selenium and present great amounts of fatty acids.[8] being indicated for the treatment of skin and eye diseases.[9] Tocopherols, phytosterols, quercetin, rutin, gallic and cafeic acids have also been previously described as chemical constituents of tucumã.[5,7,10, 11] Due to its high nutritional value and pleasant taste, tucumã fruits are widely consumed by local population as food, being used *in natura* andto produce ice cream, dessert, cakes and sandwiches.[12,13] Tucumã fruit is a typically Amazonian raw material, which can be used for extraction of an oil with great economic importance.[1,14] Oleic acid is a fatty acid constituent of this oil, however, carotenoids develop a main role as bioactive substances, being b- carotene the major substance. This substance is a pro-vitamin A and found in tucumã in higher concentrations than in carrot.[10,13]

Nanotechnology involves manipulation of materials and structures in a nanometric scale, being considering a promising tool to develop innovative pharmaceutical, cosmetics and food products.[15] Concerning food nanotechnology, studies have focused mainly to food packaging.[16] or development of a wide range of nano formulations.[17-19] On this context, nanoemulsions appear as promising food products.[20] They have small droplets ranging from 30-300 nm.[21] which are associated to kinetic stability of these systems, being also associated to increased absorption and potential bioavailability of substances.[22] Despite great potential of tucumã, to our knowledge, its fruits remain unexplored concerning development of a nanobiotechnology product. The aim of the present study was to develop nanoemulsions containing tucumã fruits oil.

Materials and methods

Chemicals

Sorbitan oleate (HLB: 4.3) and Polysorbate 80 (HLB: 15) were purchased from Praid Produtos Quimicos Ltda (São Paulo, Brazil). *Astrocaryum aculeatum*ripe fruits were harvested in Cayenne (French Guyane) and identified by Dr. Didier Bereau.

Tucumã oil extraction

30 g of fresh pulp of tucumã were extracted with 300mL of cyclohexane using a Soxhlet apparatus for 2 hours. Further removement of organic solvent was performed using a rotary evaporator under 35 °C. Tucumã oil was stored in amber glass flask under 4°C until it was used for nano emulsion preparation.

Emulsification method

Emulsions were prepared by phase inversion technique with some modifications [24]. Required amounts of both emulsifiers were dissolved in tucumã oil and heated at 65±5°C. Aqueous phase was separately heated at 65±5°C. When both phases reached the same temperature, aqueous phase was continuously added through the oil phase. The mixture was submitted to magnetic agitation for 10 min, furnishing a primarily emulsion. Final homogenization was achieved using a T25 Ultra-Turrax homogenizer (Ika-Werke, Staufen, Germany) equipped with a 25 N-18 G disperser for 5 min (8000 rpm).

Required HLB determination of tucumã oil

Each emulsion was prepared at a final mass of 25 g, containing 90 % (w/w) of distilled water, 5% (w/w) of tucumã oil and 5% (w/w) of a mixture of emulsifiers [23-24]. Series of emulsions were prepared using sorbitan monooleate as most hydrophobic emulsifier, and polysorbate 80 as most hydrophilic emulsifier. HLB values ranging from 4.3 (5% w/w of sorbitan oleate) to 15 (5% w/w of polysorbate 80) were prepared by blending together emulsifiers in different ratios. Required HLB value of tucumã fruits oil was determined as the HLB of surfactant or surfactants mixture which was able to achieve the most stable formulation.

Characterization of emulsions

Formulations were characterized after 1 and 7 days of manipulation. Macroscopical aspects (appearence, translucence, opacity, bluish reflect, phase separation, creaming and sedimentation) were examined. Polidispersity and mean droplet size were determined by photon correlation spectroscopy using a Zetasizer 5000 (Malvern Instruments, Malvern, UK). Each emulsion was diluted using ultra-pure Milli-Q water (1:25). Measures were performed in triplicate and average droplet size was expressed as the mean diameter.

Results and discussion

Studies focusing on HLB determination of oils are very important if development of emulsions is desired and is considered a valuable tool during development stage.[25] including for nanoemulsions. This can be obtained by calculation the HLB value of a single surfactant or a blend of surfactants which induce formation of the most stable emulsion, among a set of formulations with different surfactant ratios in a wide range of HLB values.[26] On this context, several emulsions were prepared using tucumã fruits oil using a pair of lipophilic (sorbitan monooleate) and hydrophilic (polysorbate 80) surfactants. Set of formulations was prepared with HLB values ranging from 4.3 (HLB of sorbitan monooleate) to 15 (HLB of polysorbate 80). Emulsions prepared with HLB of 4.3,5,6,7,8 and 9 presented milky aspects, besides signals of instable behavior, including high levels of creaming and phase separation. Moreover, mean droplet size above 300 nm and high polidispersity (data not shown) suggested absence of kinetic stability, which is an intrinsic characteristic of nanoemulsions.[27]

Formulation prepared with HLB of 10 presented mean droplet size below 300 nm (282.9±8.266 nm) after one day of preparation, however, this size increased to 312.6±5.609 nm after seven days of storage. Polidispersity also increased, revealing a polimodal distribution. Coalescence of the droplets may be attributed to these effects, being associated to the inability of surfactant to absorb to the interface.[28] It was observed a decrease in the mean droplet size of formulations prepared with surfactants at HLB values of 11, 12 and 13. They ranged from 305.6±5.782 (HLB 11), 233.4±3.032 nm (HLB 12) and 198.9±1.601 (HLB 13) after 1 day of preparation to 211.5±6.825 (HLB 11), 190.1±7.736 (HLB 12) and 156.6±0.6557 (HLB 13) after 7 days of preparation. Utilization of volatile organic solvent may be associated to this phenomenon, considering that evaporation of residual solvent during storage may decrease droplet diameter.[20] Mean droplet size for formulation with HLB of 14 increased (219.8±11.20 nm after 1 day and 236.5±7.317 nm after 7 days). Considering that polidispersity did not present significant variation, this increase may be associated to destruction and regeneration of the micelles.[29]

Additional formulations were prepared with HLB values close to 13, which presented the smallest droplet size (156.6±0.6557 nm) and polidispersity (0,240±0,006) after seven days of storage. Mean droplet size analysis revealed that these formulations presented values between 300-200 nm after 7 days of preparation, being also characterized as nanoemulsions. Mean droplet size and polidispersity are presented in Table 1.

Formulation in which HLB value of surfactants coincide with required HLB of the oil can be determined, considering that it should present the smallest mean droplet among a set of emulsions prepared with different ratios of a pair of surfactants.[23] On this context, it was possible to determine tucumã fruits oil at HLB 13. Mean droplet size distribution and polidispersity index is shown at Figure 1. Determination of required HLB value of oils has been successfully used to achieve small droplets in a nano size range.[23,24,30]

Table 1 Mean droplet size and polidispersity of emulsions prepared during required HLB determination of tucuma fruits oil

HLB	Mean diameter (nm) ± SD 1 day	Polidispersity ± SD 1 day	Mean diameter (nm) ± SD 7 days	Polidispersity (nm) ± SD 7 days
10	282.9±8.266	0.581±0.079	312.6±5.609	0.728±0.026
11	305.6±5.782	0.685±0.047	211.5±6.825	0.406±0.013
12	233.4±3.032	0.443±0.009	190.1±7.736	0.388±0.010
12.25	197.8±41.158	0.364±0.016	235.4±6.525	0.443±0.025
12.50	199.3±80.57	0.586±0.046	220.6±3.479	0.390±0.013
12.75	301.3±54.82	0.726±0.104	219.8±2.401	0.410±0.008
13	198.9±1.601	0.377±0.024	156.6±0.6557	0.240±0.006
13.25	220.3±7.481	0.522±0.090	220.3±7.481	0.522±0.090
13.5	176.0±8.107	0.426±0.020	264.1±2.991	0.670±0.008
13.75	183±3.620	0.433±0.044	250.5±11.76	0.741±0.036
14	219.8±11.20	0.662±0.033	236.5±7.317	0.665±0.073

HLB Balance hydrophilic-lipophilic; nm: nanometer

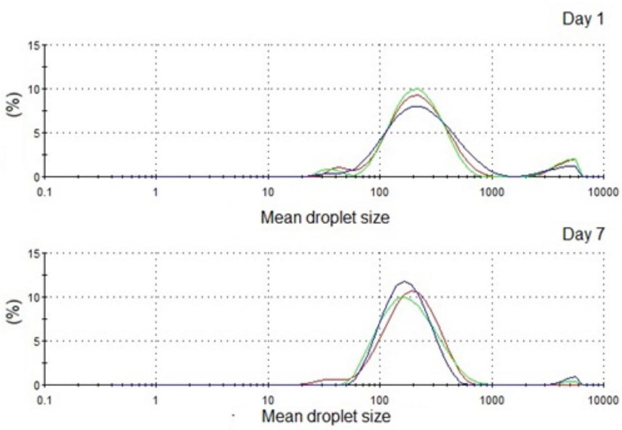

Figure 1 Mean droplet size and polidispersity of nanoemulsions prepared with 5% (w/w) of tucuma oil, 5% (w/w) of surfactants (HLB 13) and 90 % (w/w) of water. Day 1: Mean droplet - 198.9±1.601 nm; polidispersity – 0.377±0.024. Day 7: Mean droplet – 156.6±0.6557 nm; polidispersity – 0.240±0.006.

On the present study, formulations at HLB 11.0, 12.0, 12.25, 12.5, 12.75, 13.25, 13.5, 13.75 and 14 were considered nanoemulsions. These are a special type of nano formulation with mean droplet ranging from 30-300 nm, being characterized by the dispersion of two immiscible liquids and presence of one or more surfactants.[21] They are also called mini emulsions.[31] and small droplets are associated to kinetic stability of these systems, being also associated to increased absorption and potential bioavailability of substances.[22] Development of nanoemulsions as enhancers of chemical stability of natural products is considered promising, allowing, protection of substances from degradation, including oxidation.[32] Moreover, nanoemulsions prepared with carotenoids have been considered potential food products and are in the spotlight of nanobiotechnology research.[20]

Conclusion

The present study allowed achievement of nanoemulsions using different blends of non-ionic surfactants. Moreover, considering the lowest mean droplet (156.6±0.6557 nm) observed after seven days of storage, it was possible to determine required HLB value of this oil (HLB 13). On this context, this study provides valuable information concerning nanobiotechnology of Amazonian oils with great interest for pharmaceutical, cosmetics and food industries.

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Conflicts of interest

None.

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