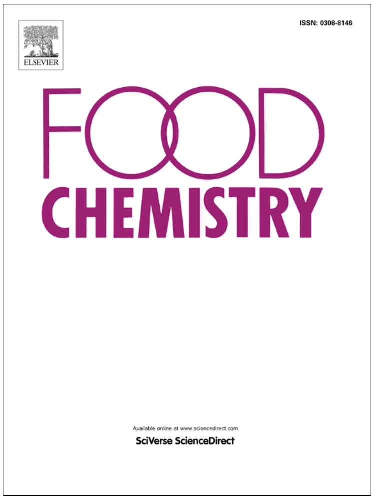

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Title page

Preparation of aqueous nanodispersions with annatto (*Bixa orellana* L.) extract using an organic solvent-free and low energy method

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ABSTRACT

The seeds of *Bixa orellana* L. is widely used in food industry as a natural colorant. A major technological challenge for its utilization on aqueous food products is the low water solubility. Therefore, the present study aimed to obtain aqueous nanodispersions using this natural raw material by using a simple organic solvent-free and low energy method. Bixin and geranylgeraniol were found on the extract. The nanodispersions were prepared by addition of water on a mixture of extract and surfactant (s). The effect of the surfactants and dilution on the nanodispersions were also evaluated. Best results were achieved using polysorbate 80/sorbitan monooleate (HLB 13) (diluted in water, 1:10). The results shows the potential of this ecofriendly approach, in contrast to common methods that make use of potentially toxic organic solvents and high input of energy, which elevate the costs for further industrialization.

Keywords: Bixin, Dynamic light scattering, Gas-chromatography couple to mass-spectrometry, Geranylgeraniol, Spectrophotometry, Thermogravimetry.

1. Introduction

Nowadays, people worldwide are interested in agents that induce less impairment to health and environment. On this context, growing interest has been observed for natural colorants, mainly due to the adverse effects of the chemicals that are used for this purpose, including potential induction of cancer, allergy and others problems. Most of them also present biological properties and substitution of synthetic chemicals by the natural products with coloring properties have been encouraged (Chengaiah et al., 2010; Shahid et al., 2013). The carotenoids develop a main role as natural colorants and have great potential for industrial applications. Among the several plants that have these compounds and high colorant potential, annatto is one of the most economic important plants (Chengaiah et al., 2010). Annatto is the common name of the species *Bixa orellana* L. Its seeds are used for obtainment of carotenoid-rich natural products that are widely used in the food industry as colorant of butter, cheese, bakery products, oils, ice creams, sausages, cereals and extruded products (Cardarelli et al., 2008).

Bixin is the most important carotenoid of annatto seeds and the coloring potential of its extract is associated to this compound (Ramamoorthy et al., 2010). However, the hydrophobicity of bixin is a major problem if solubilizing in water is required. Some strategies have been used to overcome this technological challenge, such preparation of water-soluble potassium carbonate or oil/water soluble propylene glycol formulations (Mala et al., 2015). The annatto seeds also have fatty acids, such as palmitic, oleic and linoleic acids (Rao et al., 2015), in addition to geranylgeraniol (Costa & Chaves, 2005), that also contributes to the low water solubility of this extract.

The preparation of nanostructures for food industry as potential delivery systems, including as enhancers of water-solubility, is considered very promising. Moreover, it offers additional advantages, including potential enhancement of bioavailability. The concept of aqueous nanodispersions containing natural products involves oil in water nanoemulsions or polymeric nanoparticles dispersed in water

(Surassmo et al., 2010; Ribeiro et al., 2008). However, the term nanodispersion is more associated to dispersed systems of non-liquid compounds on water and without any coating polymer (Tan & Nakajima, 2005; Anarjan et al., 2010; Leong et al., 2011; Silva et al., 2011; Shariffa et al., 2016; Tan et al., 2016a,b,c). The aqueous nanodispersions are colloidal systems containing dispersed particles with average size bellow 1000 nm. However, the issue related to the exact mean diameter that should be used to define nanodispersions is still unclear. Several upper limits are accepted according to author criteria (eg. 500, 300, 200 and 100 nm). It should be highlighted that no major physical-chemical alterations may occur when it is below 1000 nm and therefore, the nanodispersion system must show a differential property to the bulk material, regarding the intended application (Solans & Solé, 2012). It is worth mentioning that, in case of parenteral nutraceuticals, the mean droplet size must be especially critical criteria due to several adverse effects and risk of larger droplets or particles. However, for food products containing nanostructures orally consumed, this would not be a major issue if the aqueous nanodispersion induce the desired characteristics, such as physical stability, improved water solubility of the encapsulated compounds and, in case of colorants, satisfactorily homogeneity and expected colour appearance due to improved dispersability of colorant nanostructured compounds, in comparison to bulk materials.

The major approaches for the preparation of nanodispersions for industrial applications are associated to utilization of organic solvent-based methods (Silva et al., 2011). The organic phase constituted by a water-immiscible solvent (eg. hexane) is associated to an emulsification-evaporation method, while an organic phase constituted by water-miscible (eg. ethanol) solvent is associated to a solvent displacement method. However, in both cases the volatile organic solvent that is used for solubilizing of entrapped compounds must be removed (Leong et al., 2011).

Low energy methods involving organic solvent displacement is considered a suitable alternative to high-energy methods. (Tan et al., 2016c). Despite the utilization of organic solvents can be considered an advantage in terms of obtainment of nano-scale dispersed system, the potential toxicity of residues even after evaporation must be considered (Kelmann et al., 2007).

Moreover, most of the reported papers in the literature make use of high input of energy together with utilization of organic solvents (Silva et al., 2011).The aqueous nanodispersions prepared with some carotenoids, such as astaxanthin, have been well investigated as promising systems for application into food matrices (Delgado et al., 2017). Thus, as part of our ongoing investigations with annatto nanodispersions, the present study aims to generate aqueous nanodispersions with annatto extract using a simple methodology without necessity of organic solvent, based on a green and ecofriendly concept.

2. Materials and methods

2.1. Plant material

Seeds of *Bixa orellana* L. were collected at the municipality of Macapá (S 00º01'07.5" W 051º06º'18.4") at Amapá State (Brazil). Identification of plant material was performed by the botanist Rosângela Sarquis and a voucher specimen is deposited at the Herbário Amapaense (HAMAB) under register number 10068.

2.2. Chemicals

The non-ionic surfactants (sorbitan monooleate, polysorbate 80 and polyethylene glycol 400 monooleate) were obtained from Praid® Especialidade Químicas (SP, Brasil). Acetone used on the extraction of *B. orellana* (annatto) seeds was purchased from Vetec® (RJ, Brasil).

2.3. Annatto extract

Extraction of annatto seeds was performed according to a method described by Costa and

Chaves (2005) with some modifications. The seeds (814.94 g) were removed from the fruits and immediately extracted with acetone by maceration. This procedure was successively performed with solvent renewal (7 L) until a clear filtrate was obtained. Extraction was performed under room temperature and protected from light. The filtrates were pooled together and concentrated under reduced pressure using a rotary evaporator IKA® (modelo HB 05). Then, the concentrated extract was lyophilized in order to remove any trace of solvent and stored in amber glass flask at 4ºC.

2.3.1. Determination of bixin content by UV/Vis spectrophotometric assay

The bixin content on the annatto extract was determined by a spectrophotometric assay (Costa & Chaves, 2005) with some modifications. Measurements were performed on an UV Mini spectrophotometer (Shimadzu, Japan) at 470 nm. It was solubilized with chloroform and bixin content was determined using the specific absorptivity coefficient of bixin (2826 L g^{-1} cm^{-1}) in chloroform using a 1 cm cuvette. The analysis was performed in triplicate and result expressed as mean ± standard deviation.

2.3.2. Infrared analysis

The annatto extract was analyzed by infrared spectroscopy using an attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectrophotometer (Shimadzu ® Corporation IR Prestige 21 Cat. No. 206-73600-36-Kyoto-Japan). Spectral region was in the range of 4000-600 cm^{-1} with resolution of 4 cm and 32 scans.

2.3.3. Thermogravimetry analysis

Annatto extract was subjected to a thermal analyzer (Shimadzu®, model TGA 50/50H). Experimental conditions were as follows: nitrogen and synthetic air atmosphere with flow rate of 50 mL/min, at a 10ºC min ratio on a temperature range of 25-600 ºC, using a platinum crucible.

2.3.4. Gas-chromatographic analysis

Annatto extract was analyzed using a GCMS-QP 2010 gas-chromatograph coupled to mass spectrometer (Shimadzu) using electron ionization (70 eV). It was solubilized in dichloromethane and experimental conditions were as follows: injector temperature, 270 °C; detector temperature, 270°C; carrier gas, Helium; flow rate 1 mL/min; split injection with split ratio 1:60. The oven temperature was programmed from 180 °C, with an increase of 10 °C/min to 270 °C, ending with a 20 min isothermal at this temperature; RTX-5MS column (i.d. = 0.25 mm, length 30 m, film thickness = 0.25 μm). Mass spectrometry (MS) conditions were ionization voltage, 70 eV and scan rate, 1 scan/s. Mass range was *m/z* from 50 to 600). Identification was performed by comparing to literature data.

2.4. Annatto aqueous nanodispersions

Stock solutions of annatto extract (0.100 g) suspended on surfactant (s) (2.9 g) were individually prepared after homogenization under magnetic stirring (400 rpm) for 2 hours. Then, the mixtures were centrifuged by 15 min and the supernatants were collected and used as oily phase for the preparation of the nanodispersions. The oily phase (2 g) was placed on a becker and distilled water (98 g) was added under controlled flow (2.8 mL/s) and constant magnetic stirring (500 rpm). Total homogenization process was performed for 1 hour. Then, annatto nanodispersions were diluted in deionized water (1:1) prior to storage.

2.4.1. Influence of different non-ionic surfactant blends on the annatto nanodispersions

Mixtures of sorbitan monooleate and polysorbate 80 were obtained at different ratios for preparation of the oily phase. Various hydrophile-lipophile balance values (HLB = 9-15) were achieved and calculated as follows:

$$\text{HLB} = \frac{\text{HLBa x ma} + \text{HLBb x mb}}{\text{ma} + \text{mb}}$$

Where HLB = hydrophile-lipophile balance of the surfactant/co-surfactant mixture; HLBa = hydrophile-lipophile value of the most hydrophobic surfactant; HLBb = hydrophile-lipophile value of the most hydrophilic surfactant; ma = mass of the most hydrophobic surfactant and mb = mass of the most hydrophylic surfactant.

2.4.2. Influence of addition of another non-ionic surfactant aqueous dispersion

The prompt prepared annatto nanodispersions were also diluted in polyethylene glycol 400 monooleate aqueous dispersion (1%, w/w) prior to storage (1:1), in order to evaluate the influence of aqueous surfactant dispersion prepared with another non-ionic surfactant on behavior of pre-formed annatto nanodispersions prepared with different blends of sorbitan monooleate/polysorbate 80.

2.4.3. Characterization of annatto-based aqueous nanodispersions

The annatto nanodispersions were characterized using a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK) equipped with a 10 mW “red” laser ($\lambda = 632.8$ nm) and samples were measured at a 90º scattering detector angle for size measurements. They were diluted with distilled water prior to measurements, which were performed at different days (Day 0, 1, 7 and 21). Average particle size, polydispersity index and zeta potential were measured in triplicate and results are expressed as mean ± standard deviation.

2.4.4. Optimal annatto aqueous nanodispersion

After evaluation of aforementioned parameters, optimal annatto aqueous nanodispersion was chosen based on the blend of sorbitan monooleate/polysorbate 80 that allowed achievement of best results. It was diluted with deionized water at different ratios (1:10, 1:20 and 1:50). Each diluted nanodispersion was hermetically stored at different conditions, being protected or unprotected from light under controlled temperature (30 ± 2 ºC). Particle size distribution and zeta potential were measured at different days (Day 0, 1, 7 and 21). The analysis was performed in triplicate and result expressed as mean ± standard deviation.

Analysis using a programmed linear ramp of temperature was also performed as follows: temperature ramp was 25 ºC to 80 ºC, increased at 5 ºC intervals (Oliveira et al., 2017). Particle growth (PG) was determined (Mehmood, 2015; Guttoff et al., 2015) during temperature increase as follows: PG = 100 x [mean particle size (t2) –mean particle size (t1)] / mean droplet size (t1), where t2 = mean particle size after temperature increase and t1 = mean particle size on initial temperature. The analysis was performed in triplicate and result expressed as mean ± standard deviation.

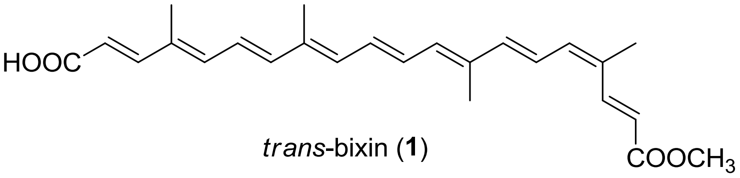
2.5. Statistical analysis

Analysis of variance (One-way Anova) with 95% of confidence interval followed by Tukey test was performed using the Software R (R Core Team, 2013). All analyses were performed in triplicate and result expressed as mean ± standard deviation. Differences were considered statistically significant when $p < 0.05$.

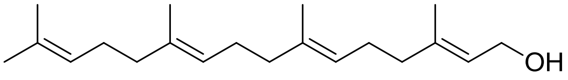
Results and discussion

Extraction of annatto seeds allowed obtainment of 31.993 g of an orange-red extract. Spectrophotometric analysis indicated a carotenoid content (expressed as bixin; w/w) of 2.09 ± 0.02 %

on the extract. This yield is in accordance with literature data for carotenoid content (expressed as bixin) of annatto extracts obtained after extraction with acetone, which ranges between 1.2 – 2.3% (w/w) (Costa & Chaves, 2005).



trans-bixin (**1**)



geranylgeraniol (**2**)

The carotenoid t*rans*-bixin (**1**) was identified on the infrared spectra (Fig. 1). The presence of characteristic peaks, such a large and intense absorption band at 3171 cm^{-1} correspondent to vibration of hydroxyl from the carbonyl group. The stretch vibration of -CH bond was observed at 2922 cm^{-1} and 2854 cm^{-1}. The absorption band characteristic for C=O bond of aliphatic α,β-unsaturated esters was also observed at 1716 cm^{-1}. The peak at 1612 cm^{-1} was attributed to asymmetric axial deformation of C=C bond of conjugated alkenes and absorption bands of C—C(C=O)—C of α,β-unsatured esters were observed within the range 1377-1159 cm^{-1}. The vibrational band at 962 cm^{-1} was attributed to methylene group of *trans*-bixin. These assignments are in accordance with literature data of this compound (Rao et al., 2014; Rahmalia et al., 2015).

Curve of mass change versus temperature (TG) were obtained to allow drawing information on thermal stability of the annatto extract. TG curve (Fig. 2) presents four events characterized by weight loss. The first event occurred in the interval of 36ºC – 50ºC with 0.4% of mass loss, that until 100ºC the mass loss corresponding to water and/or volatiles evaporation from the annatto extract. The second event of the occurred at the range of 179°C - 222°C with 27.8 % mass loss corresponding to start thermal degradation of the sample. The third event is observed at the interval 265°C-310°C with 31.9% mass loss of the materials. According to literature a temperature range of 155 °C to 310 °C corresponds to the stage of thermal decomposition of carbohydrates and other organic compounds present in the species (Costa et al., 2013). From 460°C to 485°C a sudden mass loss (8.9%) of the annatto extract occurred, indicating the carbonization of the material. However, until 179°C the annatto extract present physical stability to be incorporated formulation. This is special important, since isomerization of bixin (*cis*-trans) occurs between 200-240 ºC (Silva et al., 2005; Marcolino et al., 2011).

INSERT FIGURES 1 AND 2 HERE

In order to detect the expected volatile compound on annatto extract, GC-MS analysis was carried out. Few compounds were able to elute under the experimental conditions, however, a remarkable peak with retention time (Rt) = 6.625 min was observed. Mass spectra of this compound revealed a fragmentation pattern with peaks at *m/z* 69 (base peak), 81, 107 and 55, which correspond to literature data of geranylgeraniol (**2**) (Teixeira, 2003). This terpenoids is the main constituent of annatto extract, being even more abundant than bixin (Costa & Chaves, 2005). Geranylgeraniol is a liquid that with intrinsic water-immiscibility, and therefore, co-generation of some nanodroplets together with nanoparticles in the annatto nanodispersions should be also considered, even if the extract that was used had a powder appearance.

Figure 3 (A, B and C) shows particle size distribution and zeta potential of annatto aqueous nanodispersions prepared with sorbitan monooleate and polysorbate 80 at different blends and diluted in deionized water. Immediately after preparation (Day 0), analysis of the nanodispersions indicated that the lowest mean droplet size were observed for nanodispersions at HLB 9 (358.9 nm ± 19.0), HLB 10 (309.6 nm ± 8.6), HLB 11 (384.9 nm ± 37.5) and HLB 13 (337.4 nm ± 64.8). The absence of major particle growth is considered a good indicative of stability of aqueous nanodispersions (Mehmood, 2015), highlighting the potential of this annatto nanodispersions. Higher mean droplet size was observed at HLB 15 (876.5 nm ± 103.8). On Day 0, lowest polydispersity index was observed for nanodispersion at HLB 13 (0.483 ± 0.04), which also presented lowest variation in this parameter during storage. Lower zeta potential values were observed for nanodispersions at HLB 12 to 9, which reached negative values below -30 mV. However, the nanodispersions at lower HLB values presented major variation during storage. Less variation of zeta potential during storage was observed for nanodispersion prepared at HLB 15 followed by nanodispersion prepared at HLB 13, which remained around – 17.5 to – 24.0 mV. This parameter is associated to surface charge of colloids and classification of stability is established according to values follows: relatively stable (±10-20 mV), moderately stable (±20-30 mV) and highly stable (> ±30 mV) (Bhattacharjee, 2016).

Combining surfactant and co-surfactant with similarities in chemical structural is considered an advantage, since interaction between then would facilitate formation of stable dispersions (Asmaoglu et al., 2016). Moreover, the resulting HLB value of the mixture can be used to predict the formation of aqueous dispersions on the present study, utilization of sorbitan monooleate and polysorbate 80 in different blends using a wide range successfully allowed generation of nanodroplets. It is well known that the main problem related the physical stability of nanodispersions is associated to Ostwald ripening. On this issue, utilization of additional less polar oils and/or more lipophilic polymeric surfactant with low water-solubility is considered a good strategy to enhance the stability of the system (Tadros et al., 2004). The less alteration on particle size observed for nanodispersions that have blends of polysorbate 80/sorbitan monooleate instead of solely polysorbate 80 may be associated to these factors. Overall, considering aspects of all analyzed parameters, the nanodispersion prepared with HLB 13 should be highlighted, since it presented low mean droplet size, lower polydispersity index and zeta potential values in accordance with moderately stable systems during storage. The nanodispersions prepared at HLB 9-11 presented higher changes in polydispersity index than nanodispersion prepared at HLB 13, reaching high values (>0.500) during storage. This upper limit is related to broad size distributions (Cheong et al., 2008). However, considering high negative zeta potential values and particle size comparable to nanodispersion prepared at HLB 13, they also can be promising for further studies aiming to refine the nanodispersions, obtain narrower particle size distributions and more stable systems that also may be applicable on food products.

INSERT FIGURE 3 HERE

Figure 3 (D, E and F) shows particle size distribution and zeta potential of annatto nanodispersions prepared with sorbitan monooleate and polysorbate 80 at different blends after dilution with polyethylene glycol 400 aqueous dispersion (1% w/w) prior to storage (1:1). Similar profile after addition of this third non-ionic surfactant were observed during storage. Comparing the particle size distribution of nanodispersions diluted with deionized water and aqueous solution of polyethylene glycol 400 (1% w/w), no statistical difference was observed on nanodispersions prepared at HLB 9, 10, 11 and 13. Statistical significant difference was observed on particle size of nanodispersion prepared at HLB 12 (Day 1; p = 0.0327), HLB 14 (Day 7; p = 0.0029 / Day 21; p = 0.0024) and HLB 15 (Day 0; p = 0.0133), while statistical significant differences on polydispersity index were observed on nanodispersions prepared at HLB 15 (Day 0; .p = 0.0133). Only the nanoemulsions prepared at HLB 11 and 15 did not present statistical significant difference on zeta potential after dilutions.

Transmission electron microscopy of nanoemulsions prepared with lutein revealed similar size of droplets, when compared to dynamic light scattering (Teo et al., 2017). Several papers aiming to generate food grade aqueous nanoformulations used only the DLS to estimate the size of the droplets and/or particles. However, it is worth mentioning that TEM is a valuable tool in order to examine the shape of the nanostructures in addition to particle size distribution determination.

Enhancement of stability of nanodispersions for food application by diluting the nanostructures solely with distilled water or with co-surfactants is a promising strategy (Guttoff et al., 2015). The effect of some components, such as glycerol (Saberi et al., 2013a), propylene glycol and ethanol on diluted nanodispersions have been evaluated and improved stability was observed (Saberi et al., 2013b). A previous study performed with annatto aqueous nanodispersions indicated that polyethylene glycol 400 allowed preparation of most stable nanodispersions, when compared to a series of nanodispersions prepared with single surfactants (Prada et al., 2016). This surfactant has not water-solubility at the same level of aforementioned substances, such as glycerol, propylene glycol and ethanol, since it spontaneously form a dispersion in water (with a great tendency to sub-micron droplets). Despite this surfactant is widely used in cosmetics, to our knowledge, more studies should be carried out with this surfactant for its utilization in nanodispersions for food products.

Thus, the formulation prepared with sorbitan monooleate/polysorbate 80 at HLB 13 and diluted with water was considered the optimal annatto aqueous nanodispersion due to well-established use of these non-ionic surfactants as food grade additives and good performance on the analyzed parameters. Figure 4 (A, B and C) shows particle size distribution and zeta potential of optimal annatto nanodispersions after different dilution ratios in water (1:10; 1:20; 1:50) protected from light. The particle size profile revealed a tendency, in all dilutions, to decrease to values close to 180 – 220 nm. (Fig. 4A). The polydispersity index of the nanodispersions diluted at 1:10 and 1:50 presented a similar profile along the storage until day 14. Their polydispersity index increased after seven days of storage. Then, a decrease was observed on nanodispersion diluted at 1:10 ratio, presenting the lowest pdi (0.432 ± 0.03) after 21 days of storage. (Fig. 4B). After reaching a minimum of zeta potential value after 14 days of storage (- 26.9 ± 0.8 mV), the nanodispersion diluted at 1:10 increased to a value around -20 mV after 21 days of storage. The zeta potential values of the nanodispersion diluted at 1:50 presented a tendency for linear decrease and reached the smallest value after 21 days (- 27.8 ± 2.0 mV) (Fig. 4C).

INSERT FIGURE 4 HERE

Similar profile was observed on particle size of the nanodispersions that were protected (Fig. 4A) or unprotected (Fig. 4D) from light. However, comparison between the analyzed parameter of nanodispersions protected and unprotected from light revealed significant difference on size of nanodispersions at diluted ratios of 1:10 (Day 14; $p = 0.0038$ / Day 21; $p < 0.0001$) and 1:20 (Day 7; $p < 0.0001$). Regarding polydispersity index, difference was only observed for nanodispersion at dilution ratio of 1:20 (Day 7; $p = 0.0006$). Only this dilution did not present statistical significant difference ($p > 0.05$) on zeta potential, which was observed on nanodispersions at dilution ratios of 1:10 (Day 21; $p = 0.0088$) and 1:50 (Day 21; $p < 0.0001$).

Figure 5 shows the macroscopical appearance after 21 days of storage of optimal annatto nanodispersions after different dilution ratios in water (1:10; 1:20; 1:50). Those protected from light maintained the initial organoleptic properties, with a remarkable orange colour and homogeneous aspect. It is more pronounced on the concentrated nanodispersion and gradually decreased as the system was diluted. The storage unprotected from light induced loss of colour in all nanodispersions, especially on the diluted systems, while the major colour intensity was observed on the concentrated nanodispersion. Highly diluted nanoemulsions may be used in food systems (Chang et al., 2015), including in commercial applications such as functional foods or beverages (Saberi et al., 2013a,b) and an appropriate concentration that allow achievement of excellent final concentrations on the food product must be considered for the nano-additive (Davidov-Pardo et al., 2015). Thus, the present optimal nanodispersion in its concentrated form may be considered promising as a natural nano-colorant for further dilution and application on highly diluted food products.

INSERT FIGURE 5 HERE

Despite comparison of appearance of nanodispersion diluted at 1:10 ratio between the two conditions suggest photodegradation and alteration on colour, a bluish reflect still can be observed after storage. This is an indicative that colloidal system still are present in the formulation, since this appearance is characteristic for nanodispersions due to Tyndall effect.

Therefore, we chosen the optimal annatto aqueous nanodispersion diluted at 1:10 ratio for evaluation of the influence of the temperature on particle size distribution and zeta potential (Fig. 6A,B,C) of this nanodispersion on the day of preparation. Lowest particle growth was achieved until 35 °C (7.2%), while higher particle growth was observed until 40 ºC (22.1 %) and 45 ºC (41.6%). After the maximum that was reached at 45 ºC (522.2 ± 25.3 nm), a tendency for linear decrease was observed until 80ºC, reaching a minimum value size of 282.2 ± 13.02 nm at this temperature. The polydispersity index presented a slight change from 25 to 30 °. Then a tendency for linear decrease was observed from 30-40 ºC, reaching a minimum ($pdi_{40ºC}$ = 0.367 ± 0.03) followed by an overall increase until 80 ºC, when it reached a value slightly lower ($pdi_{80ºC}$ = 0.446 ± 0.03) than the initial polydispersity index. Changes in particle size distribution may affect the polydispersity index, if a particle size population increases or decreases. This may be induced by rearrangement of particles due to release of some entrapped compounds or even alterations due to plasticity of the film formed by the surfactants. The zeta potential value remained around -17 mV to – 15 mV until 50 ºC, reaching the value -12.7 ± 0.6 mV at 60 ºC. Then, a rapid increase was observed, ending with zeta potential value of 5.12 ± 0.9 mV at 80 ºC. Slight particle growth (<10 %) together with acceptable polydispersity index suggest relative stability (Guttoff et al., 2015), while highly unstable systems are observed when zeta potential values are ± 0-1 mV (Bhattacharjee, 2016). Thus, considering this results, we can suggest that optimal storage conditions were observed on the temperature range of 25 to 35 ºC, due to maintenance of droplet size and zeta potential values according to relative stability. However, due to relative stable behavior regarding zeta potential, further strategies aiming to generate narrower particle size distribution without any major particle growth could be performed for enhanced activity at least to 60 ºC.

INSERT FIGURE 6 HERE

Under optimal conditions, an experimental polydispersity index value around 0.410 was considered appropriate for astaxanthin aqueous nanodispersions. The method that was employed was based on the dispersion of an organic phase constituted by a dichloromethane solution of the carotenoid and its further addition to the aqueous phase under high-pressure homogenization. Considering that, the method also involved evaporation of the organic solvent prior to achievement of the final nanodispersion, it was proposed as a food ingredient (Anarjan et al., 2010). Lycopene aqueous nanodispersions were also prepared by this approach (high-pressure method and dichloromethane as organic phase constituent). Poorly dispersability was related to systems prepared solely with polysorbate 80, evidenced by pdi values greater than 0.700 (Shariffa et al., 2016). Phytosterol-based nanodispersions were also prepared. At atmospheric pressure, the utilization of hexane as component of the organic phase induced formation of internal phase with average size around 150 nm and pdi around 0.430. Polydispersity values below 0.700 were also considered acceptable for food applications by these authors. On another hand, utilization of water-miscible organic solvents as constituents of organic phase induced higher average size, highlighted by the system prepared with acetone (size around 280 nm) (Leong et al., 2011). Regarding particle size, it was observed that lutein nanodispersions (also prepared with acetone), presented higher diameter after utilization of high-pressure valve homogenization (236.6 ± 10.0 nm). At the experimental organic-phase-to-aqueous-phase volume ratio that was used (3:7), lutein nanodispersion could not be produced using the solvent displacement method. Even through some particle size larger than 200 nm, the carotenoid-based dispersed systems were considered nanodispersions (Tan et al., 2016c).

Microparticles prepared with annatto seeds (geranylgeraniol-rich oil) for food applications were prepared using ultrasonication in order to reduce the superficial diameter. The lowest mean size that was achieved was around 700 nm (Silva et al., 2015). Polymeric nanocapsules containing bixin were prepared using interfacial deposition of preformed polymers. The spontaneous formation of nanocapsules in this procedure required utilization of organic solvents. Mean droplet size was around 200 nm and the zeta potential values ranged, approximately, from -15 mV to -26 mV during storage (Lobato et al., 2013). To our knowledge, the only study regarding preparation of nanostructure systems with annatto seeds extract was carried out using single surfactants and organic solvent method. On this study, several annatto nanodispersions were prepared using single surfactants solutions as distinct aqueous phases. The organic phase was constituted by a solution of the annatto extract solubilized in acetone (Prada et al., 2016). On this context, the present study brings a substantial innovation for systems with application for food industry, since we do not use any heating step of the aqueous phase, neither organic solvents. Moreover, we use blends of non-ionic surfactants, which contributes to a better stabilization of nanostructures.

Even if the concern regarding mean diameter is of great interest, conventional emulsions prepared with carotenoids are of great interest, since several types of dispersed systems, including the conventional emulsions, are still being reported as innovative formulations for delivery of these bioactive compounds (Weigel et al., 2018). In our case, the achieved mean droplet size would not be a problem, even some of them are above 200 nm. This fact could be a problem if a parenteral nutraceutical is desired. On another hand, for food drinks it would not be a limit point, since the preparation allowed “an intended application (Solans & Solé, 2012)”, on this case, the better dispersability of the annatto extract in aqueous media.

Food grade nanoemulsions were prepared with olive oil, polysorbate 20 (surfactant) and aqueous solutions of phenolic acids (vanillic, cafeic or syringic acids) as aqueous phase. The nanoemulsions were prepared by addition of the aqueous phase through the oily phase (oil and surfactant). The mean droplet size achieved were around 250 – 500 nm (Katsouli et al., 2017). On the present study, we used low stirring speed and low cost equipment, when compared to high-speed homogenizers. However, overall, the droplet size range was similar. On another study performed with the endogenous compounds of olive oil, all formulations with mean droplet diameter below 500 nm were considered nanoemulsions. It was also observed that lower size was achieved when higher polysorbate 20 levels were used (Polychniatou and Tzia, 2018). The strategy of using higher level of surfactant, even if involve additional costs with raw material, may be an interesting approach for further studies aiming to generate annatto nanodispersions with lower size.

A study allowed achievement of β-carotene based nanoemulsions after passing the coarse emulsions through a microfluidizer. The mean droplet ranged from 146 to 415 nm as function of the oily phase composition (medium-chain triglyceride : long-chain triglyceride), respectively, from 0 to 100%. Despite the utilization of higher percentage of LCT on the oil phase induced higher droplets, the bioaccessibilty of the carotenoid increased (from 14 to 86%) after increasing the LCT percentage, respectively, from 0 to 100%. The zeta potential values were around -4.7 to -5.8 mV (Salvia-Trujillo et al., 2013). Despite we opted for a strategy using low energy method, without high input of energy, our results show particle size in accordance with the literature data, however, more satisfactorily zeta potential values. Moreover, considering that literature data revealed better bioaccessibility of a carotenoid on nanoemulsions with larger droplets, the issue related to the mean droplet size above 200 nm may not be a critical point.

Lycopene-enriched tomato extract nanoemulsions and lycopene nanoemulsions were obtained by an emulsification-evaporation technique using high-pressure homogenization followed by the organic solvent (ethyl acetate) removal. The utilization of a homogenization pressure of 80 MPa and 3 cycles of homogenization allowed achievement of droplets around 222 nm and 290 nm, respectively, for extract or lycopene-nanoemulsions (Ha et al., 2015). Despite these authors showed that high homogenization pressure and/or cycles allowed reduction of size, the overall high-input of energy required on the process and utilization of ethyl acetate may be a concern, in terms of costs or sustainability.

An aqueous nanosuspension of amorphous curcumin was prepared by a bottom-up approach. Few steps was used, where the curcumin was solubilized in ethanol (solvent), with further homogenization of this organic solution with water (antisolvent) and further removal of the ethanol and achievement of the nanosuspension with particles around 220 nm (Adityia et al., 2017). Despite the ethanol was considered a food grade solvent used in the process, the evaporation step and may be a critical point for industry. On this context, considering that the optimal nanoemulsion of our study (sorbitan monooleate/polysorbate 80 at HLB 13) diluted with water also have droplets around this value (180 – 220 nm).

Overall, the results obtained on the present study show the suitability of the present approach to generate nanodroplets with polydispersity index, in accordance with literature data for aqueous dispersed systems based on natural products that were proposed as food additives.

Conclusions

Most of studies that generated nanodispersions for application in food science makes use of organic solvents on the preparation of organic phase prior to homogenization step. Despite a further removal step is performed, a method that avoid the needs for organic solvents that may be potentially toxic in some level should be encouraged. We provided a simple method that meets this concept and make use of low energy method and low-cost equipment, which is also an advantage in terms of industrial application and consumer expectance. It is also in accordance with green and ecofriendly concepts, generating less organic solvent residues. This approach, together with the utilization of pair of non-ionic surfactants on a wide range of HLB values has been widely used for preparation of aqueous nanoemulsions. However, to our knowledge, until this moment it has not been explored in terms of aqueous nanodispersions based on solid substances or extracts. The promising annatto nanodispersion that was prepared opens perspectives for its evaluation and utilization in food matrices. Moreover, this method may be useful for other carotenoids or extracts and we believe that it open perspectives its study raw materials of great interest.

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Conflict of interest

All authors declare no conflict of interests.

Ethical approval

Not applicable.

Informed consent

Not applicable.

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References

Aditya, N. P., Hamilton, I. E., & Norton, I. T. (2017). Amorphous nano-curcumin stabilized oil in water emulsion: Physico chemical characterization. *Food Chemistry*, 224, 191-200.

Anarjan, N., Mirhosseini, H., Baharin, B. S., & Tan, C. P. (2010). Effect of processing conditions on physicochemical properties of astaxanthin nanodispersions. *Food Chemistry*, 123, 477-483.

Asmaoglu, S., Gündüz, G., Mavis, B., & Çolak, U. (2016). Synthesis and characterization of multi-hollow opaque polymer pigments. *Journal of Applied Polymer Science*, DOI: 10.1002/APP.43696.

Bhattacharjee, S. (2016). DLS and zeta potential – What they are and what they are not? *Journal of Controlled Release*, 235, 337-351.

Cardarelli, C. R., Benassi, M. de T., & Mercadante, A. Z. (2008). Characterization of different annatto extracts based on antioxidant and colour properties. *Food Science and Technology*, 41, 1689-1693.

Chang, Y., McLandsborough, L., & McClements, D. J. (2015). Fabrication, stability and efficacy of dual-component antimicrobial nanoemulsions: Essential oil (thyme oil) and cationic surfactant (lauricarginate). *Food Chemistry*, 172, 298–304.

Chengaiah, B., Mallikarjuna Rao, K., Mahesh Kumar, K., Alagusundaram, M., & Madhusudhana Chetty, C. (2010). Medicinal importance of natural dyes – a review. *International Journal of*

PharmTech Research, 2, 144-154.

Cheong, J. N., Tan, C. P., Man, Y. B. C., & Misran, M. (2008) α-tocopherol nanodispersions: preparation, characterization and stability evaluation. *Journal of Food Engineering*, 89, 204-209.

Costa, C. L. S., & Chaves, M. H. (2005). Extração de pigmentos das sementes de Bixa orellana L.: uma alternativa para disciplinas experimentais de química orgânica. *Quimica Nova*, 28, 149-152.

Costa, R. S., Negrão, C. A. B., Camelo, S. R. P., Ribeiro-Costa, R. M., Barbosa, W. L. R., Costa, C. E. F., Silva-Júnior, J. O. C. (2013). Investigation of thermal behavior of Heliotropium indicum L. lyophilized extract by TG and DSC. *Journal of Thermal Analysis and Calorimetry*, 111,1959–1964.

Davidov-Pardo, G., Joye, I. J., McClements, D. J. (2015). Encapsulation of resveratrol in biopolymer particles produced using liquid antisolvent precipitation. Part 1: Preparation and characterization. *Food Hydrocolloids*, 45, 309 – 316.

Delgado, A. M. A., Khandual, S., & Villanueva, S. (2017). Chemical stability of astaxanthin integrated into a food matrix: effects of food processing and methods for preservation. *Food Chemistry*, 225, 23-30.

Guttoff, M., Saberi, A. H., & McClements, D. J. (2015). Formation of vitamin D nanoemulsion-based delivery systems by spontaneous emulsification: Factors affecting particle size and stability. *Food Chemistry*, 171, 117-122.

Ha, T. V. A, Kim, S. Choi, Y., Kwak H.S., Lee, S. J., Wen, J., Oey, I., & Ko, S. (2015) Antioxidant activity and bioaccessibility of size-different nanoemulsions for lycopene-enriched tomato extract. *Food Chemistry, 178,115-121*.

Katsouli, M., Polychniatou, V., & Tzia, C. (2017). Influence of surface-active phenolic acids and aqueous phase ratio on w/o nano-emulsions properties; model fitting and prediction of nanoemulsions oxidation stability. *Journal of Food Engineering*, 214, 40-46.

Kelmann, R. G., Kuminek, G., Teixeira, H. F., & Koester, L. S. (2007). Carbamazepine parenteral nanoemulsions prepared by spontaneous emulsification process. *Pharmaceutical Nanotechnology*, 342, 231-239.

Leong, W. F., Lai, O. M., Long, K., Man, Y. B. C., Misran, M., & Tan, CP. (2011). Preparation and characterization of water-soluble phystosterol nanodispersions. *Food Chemistry*, 129, 77-83.

Lobato, K. B. S, Paese, K., Forgearini, J. C., Guterres, S. S., Jablonski, A., & Rios, A. O. (2013). Characterisation and stability evaluation of bixin nanocapsules. *Food Chemistry*, 141, 3906–3912.

Mala, K. S., Rao, P. P., Prabhavanthy, M. B., & Satyanarayana, A. (2015). Studies on application of annatto (*Bixa orellana* L.) dye formulations in dairy products. *Food Science and Technology*, 52, 912-919.

Marcolino, V. A., Zanin, G. M., Durrant, L. R., Benassi, M. T., & Matioli, G. (2011). Interactions of curcumim and bixin with β-cyclodextrin: complexation methods, stability and applications in food.

Journal of Agricultural and Food Chemistry, 59, 3348–3357.

Mehmood, T. (2015). Optimization of the canola oil based vitamin E nanoemulsions stabilized by food grade mixed surfactants using response surface methodology. *Food Chemistry*, 183, 1-7.

Oliveira, A. E. M. F. M. O., Duarte, J. L., Cruz, R. A. S., Conceição, E. C., Carvalho, J. C. T., & Fernandes, C. P. (2017). Utilization of dynamic light scattering to evaluate *Pterodon emarginatus* oleoresin-based nanoemulsion formation by non-heating and solvent-free method. Brazil Journal Pharmacology, http://dx.doi.org/10.1016/j.bjp.2016.11.005.

Polychniatou, V., & Tzia, C. (2018). Evaluation of surface-active and antioxidant effect of olive oil endogenous compounds on the stabilization of water-in-olive-oil nanoemulsions. *Food Chemistry*, 240, 1146–1153.

Prada, A. L., Bitencourt, A. P. R., Amado, J. R. R, Cruz, R. A. S., Carvalho, J. C. T., & Fernandes, C.P. (2016). Development and characterization of *Cassia grandis* and *Bixa orellana* nanoformulations. *Current Topics in Medicinal Chemistry*, 16, 2057-2065.

Rahmalia, W., Fabre, J. F., & Moulongui, Z. (2015). Effects of cyclohexane/acetone ratio on bixin extraction yield by accelerated solvent extraction method. *Procedia Chemistry*, 14, 455 – 464.

Ramamoorthy, S., Doss, F. P., Kundu, K., Satyanarayana, V. S. V., & Kumar, V. (2010). Molecular characterization of bixin – an important product. *Industrial Crops and products*, 32, 48-53.

Rao, M. P., Manjunath, K., Bhagawati, S. T., & Thippeswamy, B. S. (2014). Bixin loaded solid lipid nanoparticles for enhanced hepatoprotection – Preparation, characterisation and in vivo evaluation. *International Journal of Pharmaceutics*, 473, 485–492.

Rao, P. P., Rao, G. N., Jyothirmayi, T., Satyanarayana, A., Karuna, M. S. L., & Prasad, R. B. N. (2015). Characterisation of seed lipids from *Bixa orellana* and *Trachyspermun*. *Journal of the American Oil Chemists' Society*, 92, 1483-1490.

Ribeiro, H. S., Chu, BS., Ichikawa, S., & Nakajima, M. (2008). Preparation of nanodispersions containing β-carotene by solvent displacement method. *Food Hydrocolloids*, 22, 12-17.

Saberi, A. H., Fang, Y., & McClements, D. J. (2013a). Effect of glycerol on formation, stability, and properties of vitamin-E enriched nanoemulsions produced using spontaneous emulsification. *Journal of Colloid and Interface Science*, 411, 105–113.

Saberi, A. H., Fang, Y., & McClements, D.J. (2013b). Fabrication of vitamin *E*-enriched nanoemulsions by spontaneous emulsification: Effect of propylene glycol and ethanol on formation, stability, and properties. *Food Research International*, 54, 812–820.

Salvia-Trujillo, L., Qian, C., Martín-Belloso, O., & McClements, D. J. (2013). Modulating b-carotene bioaccessibility by controlling oil composition and concentration in edible nanoemulsions. *Food Chemistry*, 139, 878–884.

Shahid, M., Shahid-ul-islam, & Mohammad, F. (2013). Recent advancements in natural dye applications: a review. *Journal of Cleaner Production*, 53, 310-331.

Shariffa, Y. N., Tan, T. B., Abas, F., Mirhosseini, H., Nehdi, I. A., & Tan, C. P. (2016). Producing a lycopene nanodispersion: The effects of emulsifiers. *Food and Bioproducts Processing*, 98, 2010-216.

Silva, M. C. D., Botelho, J. R., Conceição, M. M., Lira, B. F., Coutinho, M. A. Dias, A. F., Souza, A. G., & Filho, P. F. A. (2005). Thermogravimetric investigations on the thermal degradation of bixin derived from the seeds of annatto (*Bixa orellana* L.). *Journal of Thermal Analysis and Calorimetry*, 79, 277–281.

Silva, H. D., Cerqueira, M. A., Souza, B. W. S., Ribeiro, C., Avides, M. C., Quintas, M. A. C., Coimbra, J. S. R., Carneiro-da-Cunha, M. G., & Vicente, A. A. (2011). *Journal of Food Engineering*, 102, 130-135.

Silva, E. K., Zabot, G. L., & Meireles, M. A. A. (2015). Ultrasound-assisted encapsulation of annatto seed oil: Retention and release of a bioactive compound with functional activities. *Food Research International*, 78, 159-168.

Solans, C., & Solé, I. (2012). Nano-emulsions: Formation by low-energy methods. *Current Opinion in Colloid interface & Interface Science*, 17, 246-254.

Surassmo, S., Min, SG., Bejrapha, P., & Choi, MJ. (2010). Effects of surfactants on the physical properties of capsicum oleoresin-loaded nanocapsules formulated through the emulsion-diffusion method. *Food Research International*, 43, 8-17.

Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. Advances in Colloid and Interface Science, 108-109, 303-318.

Tan, C. P., & Nakajima, M. (2005). Beta-carotene nanodispersions: preparation, characterization and stability evaluation. *Food Chemistry*, 92, 661-671.

Tan, T. B., Yussof, N. S., Abas, F., Mirhosseini, H., Nehdi, I. A., & Tan, C. P. (2016a). Forming a lutein nanodispersion via solvent displacement method: the effects of processing parameters and emulsifiers with different stabilizing mechanisms. *Food Chemistry*, 194, 416-423.

Tan, T. B., Yussof, N. S., Abas, F., Mirhosseini, H., Nehdi, I. A., & Tan, C. P. (2016b) Stability evaluation of lutein nanodispersions prepared via solvent displacement method: the effect of emulsifiers with different stabilizing mechanisms. *Food Chemistry*, 205, 155-162.

Tan, T. B., Yussof, N. S., Abas, F., Mirhosseini, H., Nehdi, I. A., & Tan, C. P. (2016c). Comparing the formation of lutein nanodispersion prepared by using solvent displacement method and high-pressure valve homogenization: effects of formulation parameters. *Journal of Food Engineering*, 177, 65-71.

Teo, A., Lee, S.J., Goh, K. K. T., & Wolber, F. M. (2017). Kinetic stability and cellular uptake of lutein in WPI-stabilised nanoemulsions and emulsions prepared by emulsification and solvente evaporation method. Food Chemistry 221 (2017) 1269–1276.

Teixeira, D. F. (2003). Estudo químico e avaliação biológica de *Attalea excelsa* Mart. ex Spreng.

(urucuri) e *Pterodon emarginatus* Vog. (Sucupira-branca) em *Aedes aegypti*. Rio de Janeiro, 154 p. Dissertação de Mestrado, Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal do Rio de Janeiro.

Weigel, F., Weiss, J. Decker, E. A., & McClements, D. J. (2018). Lutein-enriched emulsion-based delivery systems: Influence of emulsifiers and antioxidants on physical and chemical stability. *Food Chemistry*, 242, 395–403.

FIGURES AND TABLES LEGENDS

Fig 1. Attenuated total reflectance Fouries transform infrared (ATR-FTR) spectra of annatto extract.

Fig 2. Thermogravimetry (TG) curve of annatto extract.

Fig 3. Characterization of annatto nanodispersions prepared with polysorbate 80/sorbitan monooleate at different hydrophile-lipophile balance (HLB). Results from nanodispersions diluted in deionized water A) droplet size (nm), B) polydispersity index and C) zeta potential (mV) or diluted in polyethylene glycol 400 (1%, w/w) aqueous dispersion D) droplet size (nm), E) polydispersity index and F) zeta potential (mV).

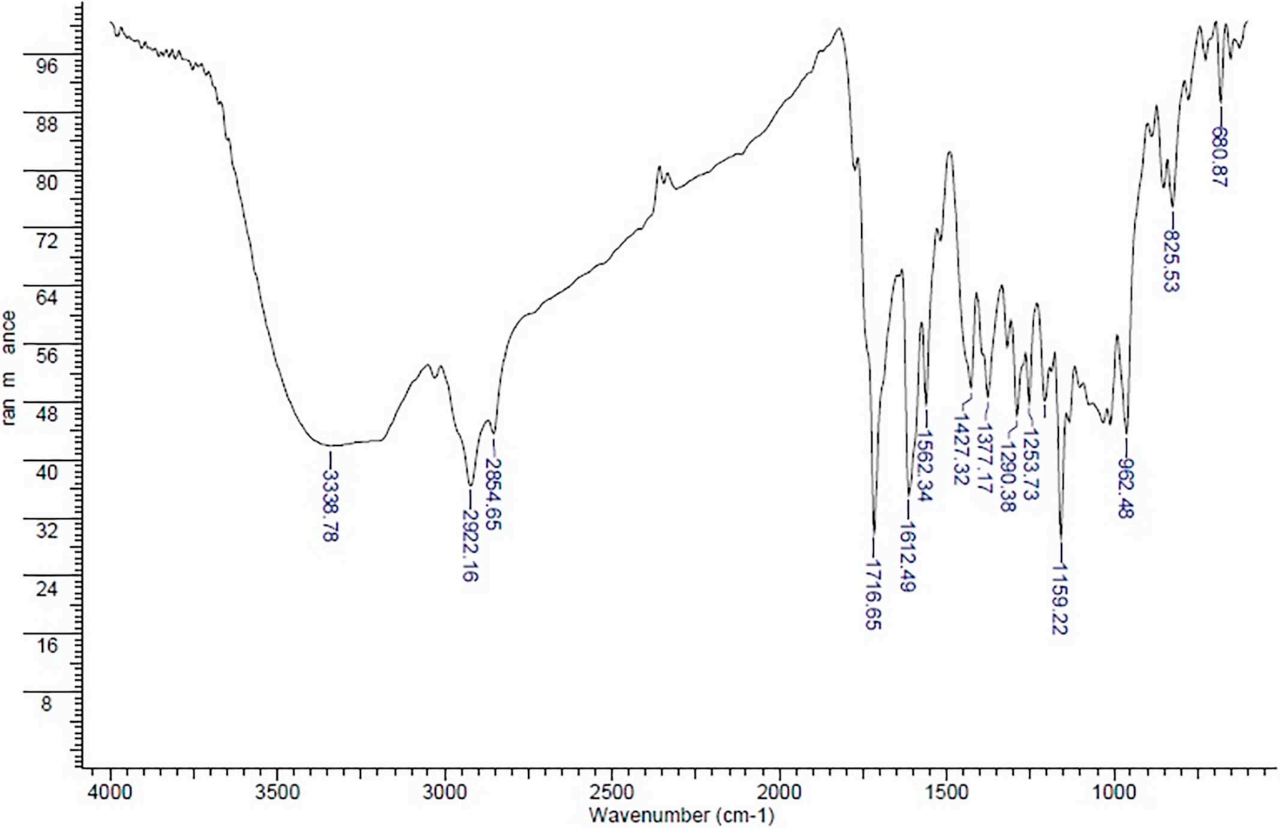
Fig 4. Characterization of optimized annatto aqueous nanodispersion (prepared with polysorbate 80/sorbitan monooleate at HLB 13) at different dilution ratios in water. Results from nanodispersions protected from light A) droplet size (nm), B) polydispersity index and C) zeta potential (mV) or unprotected from light D) droplet size (nm), E) polydispersity index and F) zeta potential (mV).

Fig 5. Macroscopical appearance of optimized annatto aqueous nanodispersion (prepared with polysorbate 80/sorbitan monooleate at HLB 13) at different dilution ratios in water (from the left to the right, respectively, concentrated nanodispersions and diluted nanodispersions at 1:10, 1:20 and 1:50) after 21 days of storage. (A) protected from light and (B) unprotected from light.

Fig 6. Evaluation of the influence of temperature increase on (A) droplet size, (B) polydispersity index and (C) zeta potential of optimized annatto aqueous nanodispersion (prepared with polysorbate 80/sorbitan monooleate at HLB 13) diluted in water (1:10).

Highlights

- Annatto is an industrial food colorant
- Its seeds extract has low water solubility
- Nanodispersions preparation often use organic solvents
- A low-energy and solvent-free method was used in this study
- The present annatto nanoemulsions is a green-product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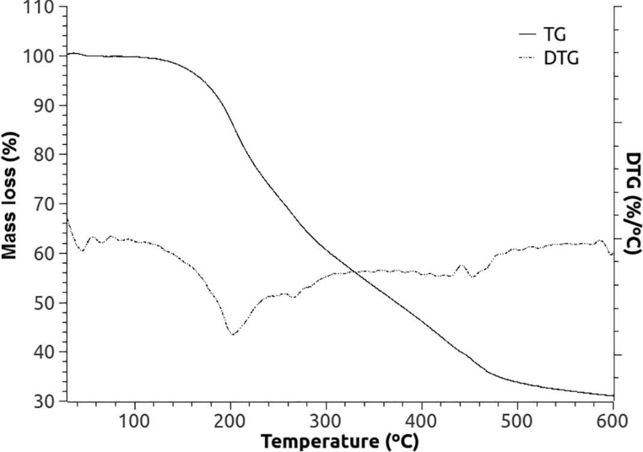
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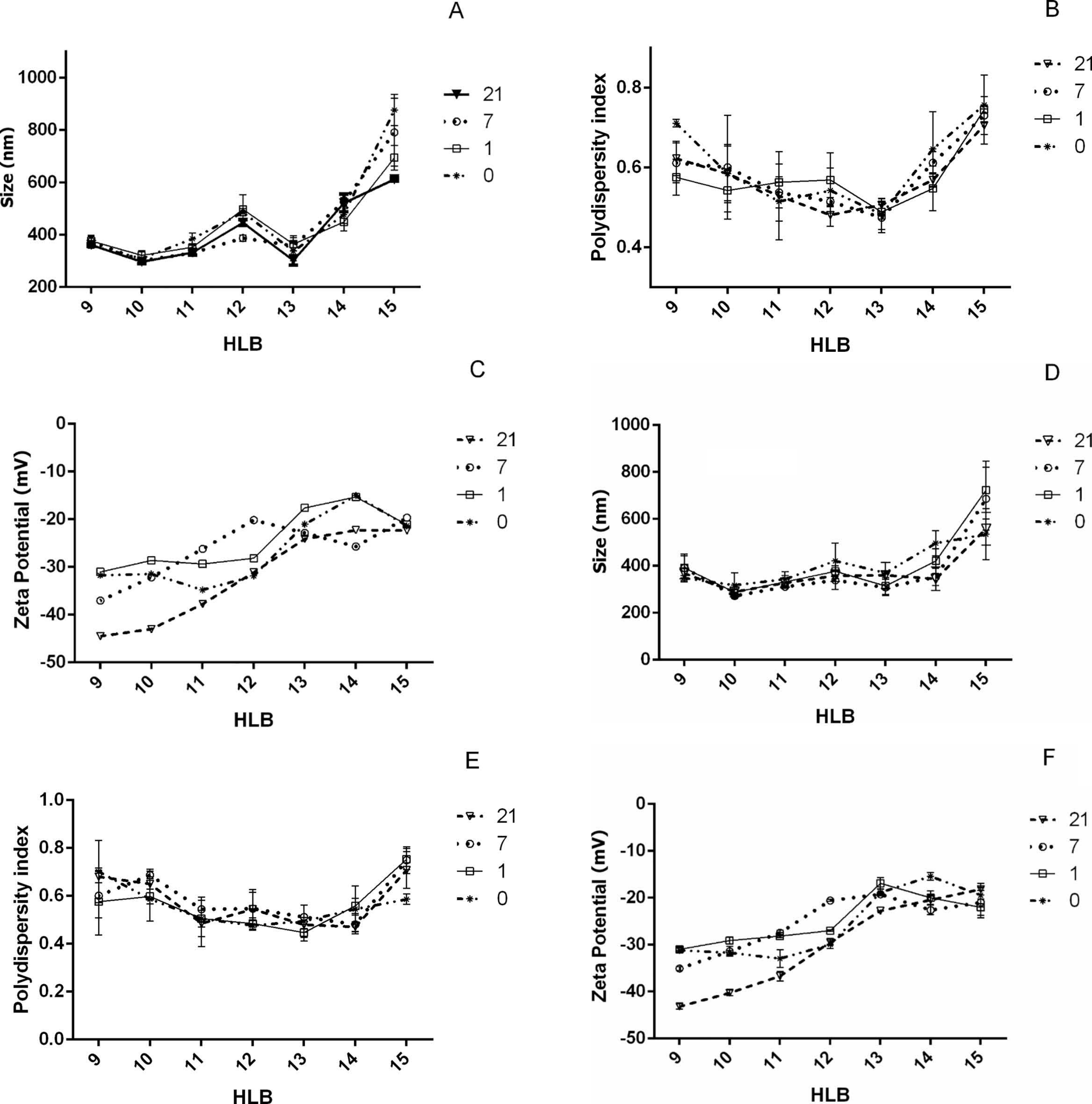
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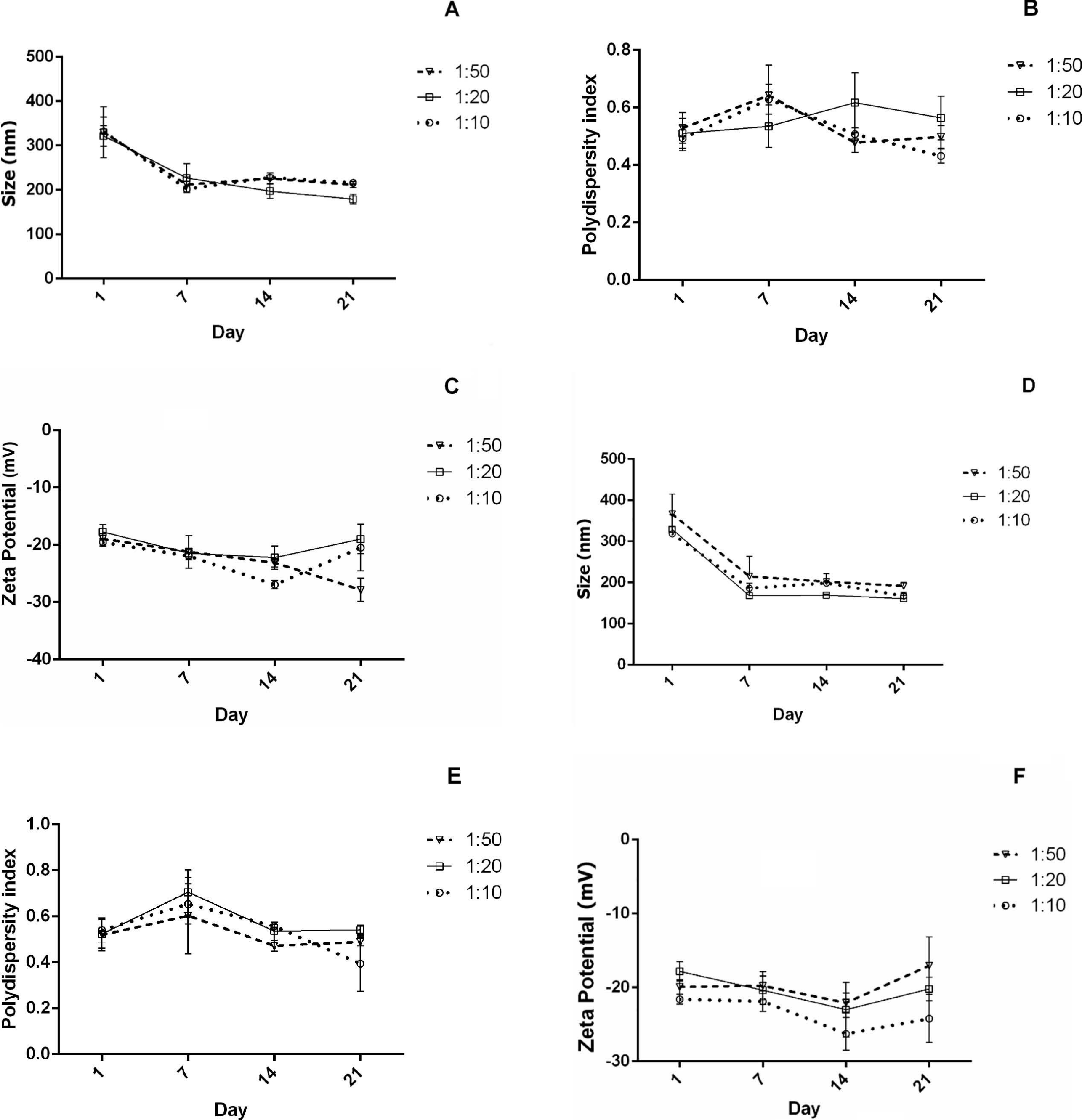
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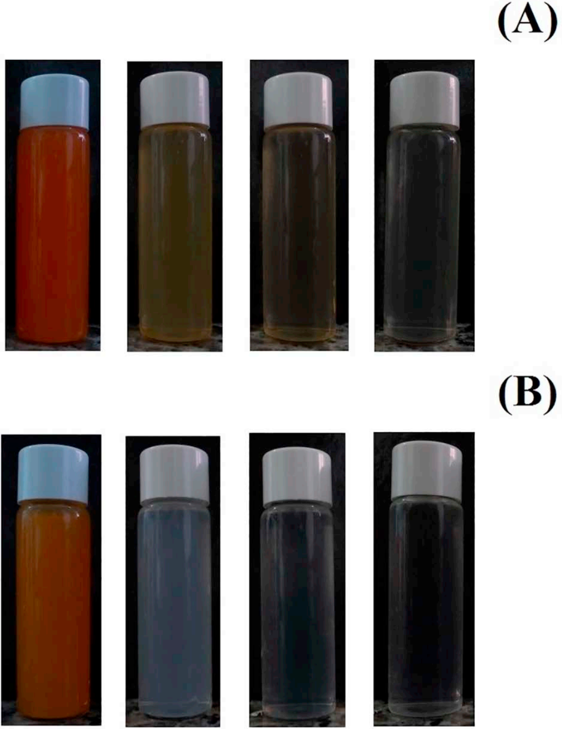
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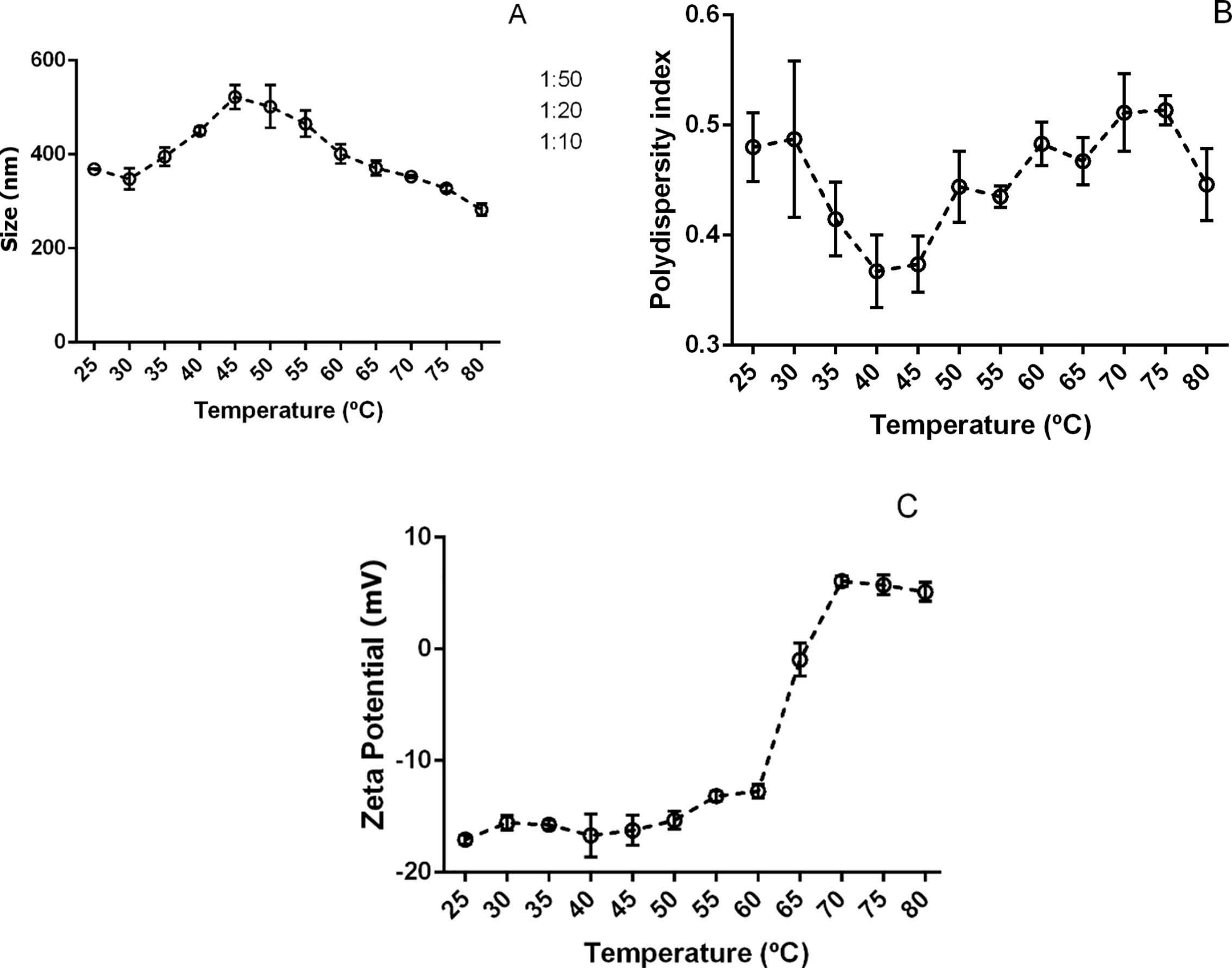
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Temperature (ºC)
B
Polydispersity index
0.3
0.4
0.5
0.6
25 30 35 40 45 50 55 60 65 70 75 80
Temperature (ºC)
C
Zeta Potential (mV)
-20
-10
0
10
25 30 35 40 45 50 55 60 65 70 75 80
Temperature (ºC)