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Preparation of surfactant-free emulsions using amaranth starch modified by reactive extrusion



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GRAPHICAL ABSTRACT



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ABSTRACT

The use of emulsions at industrial scale involves simple, efficient and versatile applications. Currently, it is preferred the exploitation of biopolymers such as modified starch that may be used as suitable wall materials, avoiding toxic surfactants during preparation. Therefore, in this work, modified amaranth starch with improved physicochemical and morphological properties was obtained by reactive extrusion from a selected extruder outlet time. Additionally, surfactant-free emulsions based on modified starch were prepared at a registered optimal time. The evolution of particle size distributions was monitored by combining Dynamic Light Scattering (DLS) and light microscopy. For starch modification, results showed that 80 s was the best outlet time from the extruder to produce a homogeneous material (particle size: 553 nm; fractal dimension: 2.73). For emulsions, a thoroughly characterisation indicated that DLS and fractal analyses converged at 270 s as optimal preparation time, showing a well-dispersed and stable system (particle size: 342 nm; fractal dimension: 2.85). Thus, this contribution provides a strategy for the obtention of modified amaranth starch having appropriate hydrophilic balance and T_g values for emulsions stabilisation. On the other hand, the technique

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https://doi.org/10.1016/j.colsurfa.2020.125550 Received 24 April 2020; Received in revised form 26 August 2020; Accepted 30 August 2020 Available online 12 September 2020 0927-7757/ © 2020 Elsevier B.V. All rights reserved. applied for emulsions preparation resulted in controllable size and shape of particles. This work proposes a complete sequential process that could be helpful to overcome the challenges faced by a number of industries.

1. Introduction

Nowadays, food and pharmaceutical fields are requiring the development of materials with enhanced bioactivity and other characteristics, including eco-friendly nature, non-toxic additives and suitable particle sizes. Thus, industries can provide advanced products that generate a positive impact on the society, attending government regulations for environmental protection. In order to obtain these materials, the use of an efficient preparation method is a key factor. Therefore, destructive or building up approaches have been proposed for particle preparation, which can be divided into several subclasses depending on reaction conditions, adopted protocols and operation. From that, low-cost chemical synthesis, low-cost operations and easily scalable processes are desired [1]. One of the objectives of particle design is the development of carriers of active substances (e.g. drugs, dyes, food additives) with industrial purposes [2]. In some cases, chemical modifications of functional groups are used to induce specific arrangements involving tailored intra- and inter-molecular interactions [3]. In this regard, systems that have been explored for this purpose are micelles, liposomes, polymeric gels and emulsions [4,5]. For instance, emulsions are a type of colloidal arrangements that consist in the dispersion of two immiscible phases (normally oil and water) commonly stabilised by using a conventional surfactant [6]. However, surfactantfree emulsion is a promising approach that can be exploited by finding the optimal hydrophobic-hydrophilic balance between the wall material and the oil phase [7,8]. Emulsions are commonly used as therapeutic systems for the delivery of bioactive substances in several human diseases like cancer, diabetes, autoimmune disorders [9]; and also, for food-grade purposes such as encapsulating antioxidants, vitamins, pigments [10]. These type of carriers present some advantages over some conventional systems, such as well-defined structure, increased bioavailability, as well as they represent a tool for targeted and combinatorial therapy [11]. However, most of these applications must be improved by studying the formation of the emulsions, starting with the selection of an adequate wall material. Hence, the importance of the wall material is clear and must satisfy characteristics such as appropriate hydrophobic-hydrophilic balance, good thermal resistance, and possess suitable functional groups. Therefore, biopolymers such as starch can represent ideal candidates for the fabrication of wall materials [12]. Native starch is an unsuitable encapsulating agent by itself and it must be modified by means of several techniques as to change original functionality [13]. Recently, some works reported that modified starch isolated from different food sources was successfully applied for emulsions fabrication [14,15]. In this sense, amaranth seeds can be a novel and promising source for obtaining modified starch since they possess starch granules with small size (1–3 $\mu m)$ and most of the required abovementioned properties, plus its biocompatibility with human physiology [16]. In spite of the aforementioned, the use of modified amaranth starch for emulsifying purposes has been slightly studied up to now. As example, Kshirsagar and Singhal [17] prepared emulsions from hydrolysed corn and amaranth starches and evaluated the effect of molar substitution on rheological properties and particle size (between 1 and 4 μ m), finding that both materials were excellent encapsulating agents for lemon oil. In another work [18], waxy corn and amaranth starches were modified by using Octenyl Succinic Anhydride (OSA), observing that both modified starches had a good emulsification capacity (75-81%), and the degree of substitution, provided by the reaction with OSA, had no significant effects on physicochemical properties of prepared emulsions. However, these modifications of amaranth starch could result in the formation of residual contaminants. In consequence, the chemical modification of starch must be carried out by means of an eco-friendly technique such as reactive extrusion which also has the advantage to produce high amounts of modified material in a short time. The reactive extrusion has been used to modify corn starches [19,20] and protein isolates [21] for encapsulating purposes. After the selection of the wall material is accomplished, it is vital to improve formulations for increasing the velocity at which emulsions are prepared, reducing costs and avoiding the use of toxic surfactants. Therefore, the formation of emulsions can be considered as the main factor to be controlled during preparation as for instance by studying the kinetics of the physicochemical and morphological properties of the resulting droplets. Hence, this work addressed the extraction of starch from amaranth seeds and its further modification by reactive extrusion, where the effect of the outlet time from the extruder on its physicochemical and morphological properties was assessed; additionally, an optimal time for the preparation of surfactantfree emulsions based on modified amaranth starch and corn oil (as oil phase model) was comprehensively investigated by Dynamic Light Scattering (DLS) and fractal analyses.

2. Materials and methods

2.1. Materials

Amaranth seeds (*Amaranthus hypochondriacus*), grown in Huazulco (Morelos, Mexico), were acquired for starch isolation. For modification of amaranth starch, glyoxal and glycerol were used as received from Sigma-Aldrich (Mexico). The emulsions preparation was performed using modified amaranth starch and commercial corn oil (MAZOLA brand, ACH Foods Mexico) that was purchased in a local market in Culiacan, Mexico.

2.2. Isolation of amaranth starch

The extraction of amaranth starch was carried out by following the methodology reported by Chandla et al. [22] with modifications. Briefly, amaranth seeds were manually cleaned, dried at 40 °C for 48 h, and milled into flour, which was passed through a 70-mesh sieve (British Standard Size). Then, flour was steeped in alkali solution (NaOH 0.0625 M) using a flour: alkali ratio of 1:5 at 5 °C for 25 h. Then, filtration was carried out using sieves (120, 200, and 300 mesh). Filtrate/permeate collected after filtration (milky starch) was obtained at a first stage, and the retained residue on screens were collected and wet-ground with fresh water (1:5) for 2.5 min. Filtration processes were repeated and filtrate (starchy milk) was collected at a second step. Subsequently, milk obtained at first and second stages were thoroughly mixed and centrifuged at 8000xg for 15 min. The supernatant was discarded, while protein layer was scraped off. The white material, that remained after the removal of protein, was collected as wet mass of starch. Wet starch was dried at 80 °C for 4 h and finally, it was milled to obtain a white powder. The proximate composition (dry basis) of this product was ash (0.1%), protein (2%), lipids (0.3%), crude fibre (0.1%), carbohydrate (97.5%) and moisture (9%).

2.3. Modification of amaranth starch by reactive extrusion

The modification of amaranth starch was based on the method reported by Song et al. [23] which consisted in performing a crosslinking chemical reaction by means of extrusion. Crosslinking involved the formation of hemiacetal bonds between the two carbonyl groups of glyoxal (crosslinker agent) and hydroxyl groups of starch molecules (Fig. 1). Briefly, the procedure started by mixing amaranth starch, water, glycerol and glyoxal (10 wt.%), obtaining a mixture with 30% of moisture content which was stored at 2 °C for 24 h. Then, the material was subjected to an extrusion process in a single screw extruder (20 DN, CW Brabender Instruments Inc, USA) at 110 °C and 240 rpm. In order to found an optimal extruder outlet time, the extrudate was collected at 5 different extruder outlet times (40, 80, 120, 160 and 200 s). The extruder outlet time was taken after the first portion of extrudate material was visible emerging from the extruder die. The properties of these five samples were compared against the native starch (0 s of extruder outlet times). Each extrudate (samples at 5 different extruder outlet times) were dispersed in water by an Ultra Turrax homogenizer (IKA, Germany) at 16 000 rpm for 30 min. Finally, the samples of modified amaranth starch were freeze-dried at -51 °C and 0.019 mbar.

2.4. Emulsions preparation

Oil-in-water emulsions from modified amaranth starch (as wall material) and corn oil (as model lipid agent) were prepared by the rotor-stator emulsification method [24] in a 2.5:1 ratio of modified starch and corn oil, respectively. The wall material consisted in a mixture of modified amaranth starches collected from the optimal extruder outlet time after the reactive extrusion (from 80 to 200 s). Initially, an aqueous solution (type I water) of modified amaranth starch was obtained, then corn oil was added to achieve a solution with 10% total solids content in 20 mL (total volume) at room temperature. The mixture was dispersed by using an Ultra Turrax homogenizer (IKA, Germany) at 13 000 rpm at 8 different preparation times (30, 60, 90, 150, 210, 240, 270 and 300 s) in order to find an optimal time for the preparation of stable emulsions having well-defined morphology and suitable particle size.

2.5. Fourier Transform Infrared (FTIR)

FTIR Spectroscopy was used to examine alterations of functional groups after the chemical modification by reactive extrusion. Data were obtained by using a Spectrum 400, FTIR/FT-NIR Spectrometer (Perkin Elmer, US) in the spectral range from 4000 to 650 cm⁻¹. The samples were analysed as dried powder and 14 scans were collected.

2.6. Thermogravimetric Analysis (TGA)

With the aim of detecting the presence of glyoxal units in modified amaranth starch after reactive extrusion, the thermal stability of native starch and crosslinked starch was assessed by TGA over a temperature range of 30–800 °C at a heating rate of 10 °C min⁻¹ under nitrogen purge. The equipment used was a SDT2960 simultaneous DSC-TGA (TA Instruments, US).

2.7. Differential Scanning Calorimetry (DSC)

The glass transition temperature (T_g) of native and modified amaranth starch were determined by analysing the thermograms obtained from a Differential Scanning Calorimeter (Perkin Elmer, USA). The scanning was performed from 25 °C to 150 °C at a heat flow of 5 °C min⁻¹.

2.8. Dynamic Light Scattering (DLS) analysis

Hydrodynamic Diameter (D_h , taken as a particle size), polydispersity index (PDI = μ_2/Γ^2 , from the cumulant method) [4] and ζ potential of amaranth starch (native and modified) and emulsions were measured using a DLS equipment (ZS90, Malvern Instrument, UK). For all samples, 1 µL was diluted in 10 mL of type I water.

2.9. Scanning Electron Microscopy (SEM)

Images of native and modified amaranth starch were acquired using a Scanning Electron Microscope (JEOL, Japan) at a magnification of 2000X, distance work: 10 mm, and a voltage of 2 kV for secondary electrons detector (LED).

2.10. Light microscopy

All emulsions samples at 8 different preparation times were observed in a light microscope (Nikon, Japan) using halogen illumination and high aperture at 40X magnification.

2.11. Fractal analysis

Fractal dimension (F_D) and lacunarity (L') are morphometric parameters that were useful to identify changes on modified amaranth starch caused by the extruder outlet time of the reactive extrusion; and the effect of preparation time of emulsions. The parameters F_D and L' were determined by means of Digital Image Analysis (DIA) which consisted in the processing of images acquired by the microscopy techniques. DIA was carried out by using the software ImageJ v.1.50d (National Institutes of Health, USA) using the plugin FracLac 2015Febb5810. The SEM images of native and modified amaranth starch were processed in the software adjusting brightness automatically and contrast; then, the images were cropped from 1280 × 1024 px² to a resolution of 120 × 960 px² and transformed from RGB format (colour) to 8-bit format (grey scale). On the other hand, the images of emulsions obtained by light microscopy were processed similarly to SEM images and no cropping was necessary.

After processing, F_D and L' were evaluated for all images (native and modified starches as well as emulsions). Thus, the determination of these parameters was based on the method proposed by García-Armenta et al. [25] in which the Box Counting method was used. This



Fig. 1. Chemical modification via reactive extrusion yielding modified amaranth starch.

method consists in plotting a number of boxes (N_r) with a defined size (r) that are required to cover completely a structure's surface. The size r varies in r^{-d_B} ; where d_B is the estimation of F_D . On the other side, L' was computed as a relation of the standard deviation and mean of grey level intensity per pixel in each box at any size (r) and orientation.

2.12. Confocal Laser Scanning Microscopy (CLSM)

A Confocal Laser Scanning Microscope (Carl Zeiss, Germany) was used to detect the encapsulation efficiency of emulsion samples. Emulsion particles were stained with Nile red (fluorochrome for lipid materials) and Nile blue (fluorochrome for starch) [26] and scanned at wavelengths of 514 nm and 633 nm, respectively. After observation and image acquisition, the encapsulation efficiency of the oil phase (corn oil) was assessed by the colocalization tool from the software included in the CLSM microscope. The colocalization method consisted in calculating the percentage of overlapped red and blue pixels in relation to the total pixels of the coloured region of the image. This percentage was considered as the efficiency of encapsulation [27].

3. Results and discussion

3.1. Modification of amaranth starch

3.1.1. Characterisation

According to the literature, acid hydrolysis, non-solvent precipitation, ultrasonication and media-milling are the main methods that have been explored for starch modification and size reduction [28]. However, reactive extrusion is a tool that has showed good results for this modification [23]. Frequently, in the extrusion process at industrial scale, the first portion of the extrudate is considered as waste, but there are no systematic studies regarding the amount of material that must be discarded. In this work, in order to obtain modified amaranth starch with improved and homogeneous physicochemical and morphological properties, the effect of the outlet time from the extruder was assessed. Table 1 shows physicochemical and fractal properties from 0 s (native amaranth starch) to 200 s of extruder outlet time. In this regard, a reduction of particle size was observed between the native (0 s) and modified (40-200 s) starches, indicating that the structure of the starch granule was disrupted by the high temperature and high shear stress at which were subjected inside the extruder. A new structure with smaller particle sizes was obtained as a result of the crosslinking chemical reaction. On the other hand, ζ potential values of modified amaranth starch dispersions were found between -20 and -11 mV, which were lower in comparison to the native starch. ζ potential describes a difference of electric potential between the particle's surface and the medium. Therefore, ζ potential data (negative or positive) near to 0 have been associated to instable systems that sediment at a certain time [10]. For modified starches obtained from 80 s of extruder outlet time, it was observed that the ζ potential started to change towards lower values, i.e. modified amaranth starch exhibited a different hydrophobic-hydrophilic balance. This result suggested the development of a tailored wall material for preparing emulsions, which resulted in a substantially different system which is fully described in Section 3.2.

On the other hand, at 80 s of extruder outlet time, a starch with significant structural changes was detected based on the values of T_g (transition in amorphous polymers), which showed similar data after this extruder outlet time (80–200 s). The native amaranth starch presented a T_g similar to other cereal-based native starches, such as corn starch (20–60 °C) and wheat starch 30–90 °C [29], and the variation in this property was not significant for the modified starch obtained at 40 s of extruder outlet time. Consequently, the native starch (0 s) and the material at 40 s of extruder outlet time had comparative thermal properties, suggesting that both materials probably possessed a similar structure. At extruder outlet times from 80 to 200 s, higher T_g values (close to 117 °C) were observed, demonstrating the modification of

native starch. According to several reports [30,31], amaranth starch has been classified as a waxy starch due to it contains a low amylose percentage (4-6%). Therefore, amylopectin chains predominate and provide a more rigid structure which is lost towards an amorphous structure when starch is subjected to reactive extrusion [32]. The higher T_g values were associated to the generation of crosslinking points that decreased the segmental chain motion and lead to more rigid polymeric materials, and therefore, higher temperature is required to make the chains flexible [33]. In the case of crosslinked starch, similar data of T_g indicated that the material obtained between 80 and 200 s had guite comparable thermal stability and crosslinking density. Similarly, structural changes observed in starch modification have been reported in a recent study obtaining a T_g of 113 °C for pea starch modified with isocyanate groups [34]. However, for other reported systems involving commercial crosslinked starches with different acetylation degree, lower T_g values were registered (~88 °C). The T_g of the modified starches was 3-6 °C lower than Tg for native cassava starch (control sample) [35]. It is known that higher T_{σ} represents an advantage related to a better performance of the wall material as stabiliser for the preparation of emulsions [28].

Additionally, morphometric parameters: F_D and L' [36] were evaluated for native and modified amaranth starch, observing a slightly decrease in F_D values with the increase of extruder outlet time. F_D values started to change at 80 s of extruder outlet time, from 2.70 to 2.73, indicating an irregular three-dimensional structure caused by the crosslinking reaction carried out inside the extruder. As well as F_D , L' is a parameter that complements the fractal analysis and is commonly used to differentiate between equal F_D values. Low values of L' are associated to a higher symmetry and vice versa. F_D values appeared to be similar among extruder outlet times, however, L' values started to decrease from 80 s towards 200 s of extruder outlet time (values around 0.1). Therefore, the chemical modification of amaranth starch induced an irregular and highly symmetric three-dimensional structure. A deeply discussion of F_D and L' is presented below in fractal analysis of emulsions (Section 3.2.3).

The crosslinking chemical modification of starch was also confirmed by SEM images (Fig. 2), where the typical polygonal shape and characteristic size (between 2–5 μ m) for native amaranth starch were observed (Fig. 2A) as reported in a study of extraction of amaranth starch [16]. At the same time, the structural change of amaranth starch, as a consequence of the reactive extrusion at 80 s of outlet time from the extruder, was observed (Fig. 2B). The modified amaranth starch presented an irregular structure with some smoothness, which were characterised by fractal parameters described in Table 1.

3.1.2. FTIR and TGA analyses

FTIR spectra for native and modified amaranth starch were obtained to detect major alterations of functional groups after the chemical modification by reactive extrusion (Fig. 3A). However, according to the wavenumber and relative transmittance, both materials showed characteristic spectra similar to those reported in the literature for native and crosslinked starches using glyoxal, suggesting that the same type of

Table 1

Properties of amaranth starch after reactive extrusion.

Extruder outlet time	Physicochemical properties			Fractal parameters		
(5)	Particle size (nm)	ζ (mV)	T _g (°C)	F _D	Ľ	
0	1860	-26.73	64.73	2.700	0.141	
40	451	-20.66	75.39	2.702	0.175	
80	553	-16.28	116.54	2.731	0.100	
120	407	-16.85	118.26	2.745	0.119	
160	307	-11.86	117.10	2.747	0.086	
200	601	-13.26	115.28	2.731	0.126	



Fig. 2. SEM images of A) native amaranth starch (extruder outlet time = 0 s) and B) modified amaranth starch (extruder outlet time = 80 s). The scale bars represent 10 μ m and 1 μ m at 2000X (A1 and B1) and 5000X (A2 and B2) magnifications, respectively.

molecular vibrations were exhibited by the two starches [37,38]. The reactive extrusion allowed the preparation of crosslinked starch particles without altering significantly the functional groups of the native starch. From the FTIR spectra, a band from 3000 to 3500 cm⁻¹ was observed and related to the stretching of O–H groups; the peak close to 2900 cm⁻¹ was associated with the molecular vibration of C–H stretching belonging to aliphatic groups; the peaks between 1300 and 1600 cm⁻¹ corresponded to C–H bending; the peak observed near 1000 cm⁻¹ was related to the C–O stretching. It can be seen a difference in relative transmittance when the signals of the O–H stretching are compared for both materials, which could indicate changes in intramolecular interactions involving hydrogen bonds because of the decrease in the polymeric chains motion after the crosslinking. According to the spectra of modified amaranth, glyoxal residues or carbonyl compounds derived from hydroxyl group (–OH) were not detected [37,38].

TGA was carried out to investigate the thermal stability of native and modified amaranth starches. The TGA thermograms showed that the degradation profiles of both samples were different from each other (Fig. 3B). According to literature [39,40], the expected weight loss curve for native starch was detected. For this sample, three major decomposition stages (I-a, II-a and III-a) were registered: the first one (from 50 to 110 °C) was attributed to evaporation of adsorbed-coordinated water and other volatiles; the second weight loss stage corresponded to the degradation of starch chains that was reached between 270 and 350 °C; and a third decomposition stage for the residual material started at about 350 °C. Thus, the mechanism of thermal decomposition of this material can be drawn using three steps: (1) physical dehydration depending on moisture content, (2) chemical dehydration and thermal decomposition, and (3) thermal reactions yielding water and other small volatile molecules [39]. On the other hand, the sample of modified amaranth starch presented a lower amount of adsorbed-coordinated water (Fig. 3B). Furthermore, this material exhibited an additional weight loss stage (I-b*, from 140 to 270 °C) as compared to native starch that corresponded to the thermal degradation reported in literature for glyoxal. This additional process may be attributed to a weight loss involving a less stable structure given the presence of hemiacetal groups, which are produced by the reaction between starch and glyoxal that represents the addition of an alcohol to an aldehyde [40]. From TGA profiles of modified starch, it can be supposed that around 75% of the weight sample corresponded to the main backbone of the starch.

3.2. Emulsions preparation

3.2.1. DLS analysis

One of the aims of this work was to analyse the evolution of the particle size, depending on preparation time, from the diffusion coefficient obtained by DLS, which is also a standard tool helpful to detect macromolecular assemblies [5]. Thus, the hydrodynamic diameter $(D_{\rm h})$ of emulsions was evaluated at 30, 60, 90, 150, 210, 270 and 300 s (Fig. 4). The preparation time had a marked effect on the homogeneity of size distributions. As it can be seen, most of the samples exhibited bimodal size distributions (smaller than 100 nm and close to 350 nm), which was related to particles having a notably different diffusion coefficient. Particles with two or more different relaxation modes coexisted in some samples of emulsions [41]. Interestingly, the particle size distribution that appears at around 100 nm shifted towards the region of smaller sizes and its intensity decreased with increasing the preparation time. These smaller particles could be formed by smaller aggregates of starch chains and residual oil. It is well known that the emulsions preparation with well-controlled average size is a laborious task [42]. However, despite the lack of surfactant, unimodal size distributions were found in the case of samples prepared at 90 and 270 s, suggesting that particles with a single diffusivity (one relaxation mode) were obtained [4]. Although, 90 s seems to be a short time to ensure a suitable preparation of this colloidal system. Therefore, DLS data from the samples prepared at 30, 90 and 270 s were more thoroughly



Fig. 3. FTIR spectra (A) and TGA thermograms (B) of native amaranth starch and modified amaranth starch. I, II and III in Fig. 3B represent different decomposition stages.

examined (Fig. 5). DLS measurements relate the Brownian motion of particles suspended into a liquid to the particle size [41]. Hence, additional information can be obtained by inspecting the correlograms, given that the signal decays more rapidly when the particle size decreases, as well as an extended decay is associated to samples with high polydispersity [43]. This data analysis contrasting the correlograms of different samples is non common when particle size distributions are assessed by DLS. The sample collected at 30 s showed a signal with a more extended decay, and the correlation of this signal decayed more rapidly as compared to samples at 90 and 270 s. For the sample at 90 s, two relaxation modes were apparently detected; however, one of them was curiously not registered in the respective particle size distribution. The additional slower relaxation mode may be related to a very low concentration of emulsion clusters [43] that were probably dismissed by the DLS software when particle size distributions were drawn. Hence, the preparation time at 270 s was selected as suitable for emulsions formation, since the corresponding sample showed a proper monoexponential relaxation time, as compared to samples produced at all the preparation times studied. Additionally, the appearance of the sample obtained at 30 s presented macroscopic aggregation, whilst the sample at 270 s exhibited an appearance of a homogeneous milky mixture.

Table 2 summarizes values of the particle size (D_h) and



Fig. 4. Particle size distributions of emulsions at different preparation times obtained by DLS.

polydispersity index (PDI) obtained from the cumulant method (PDI = μ_2/Γ^2), as well as ζ potential determined by DLS. In the case of samples showing a bimodal size distribution, both mean sizes showed differences larger than 2-fold between them. PDI values were within the range of 0.434 – 0.713. When comparing the PDI of samples prepared at 90 and 270 s, both had similar D_h and unimodal size distribution. Thus, it was evident that a longer preparation time allowed the formation of



Fig. 5. Representative correlograms from emulsions obtained at three preparation times: a) 30 s, b) 90 s and c) 270 s.

Table 2

Data	from	Dynamic	Light	Scattering	(DLS)	of	emulsions.
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Preparation time (s)	D_h^* (nm)	PDI (μ_2/Γ^2)	ζ (mV)
30	105; 396	0.434	- 8.36
60	59; 342	0.668	-7.31
90	342	0.713	-7.40
150	38; 430	0.458	-6.86
210	50; 430	0.588	-6.61
240	59; 430	0.524	-7.98
270	342	0.555	-7.70
300	68; 430	0.573	-5.77

* Samples showing two values, separated by a semicolon, presented bimodal distributions.

more homogeneous particles (lower PDI), being that in agreement with the analysis presented above about the suitable preparation time (270 s). As expected, in the case of ζ potential values, no effect of the preparation time on the charge of particle's surface was registered, given that the same wall material, oil phase and aqueous medium (same pH) were used for preparing all samples. It is noteworthy that when comparing particle size of emulsions and modified amaranth starch (Table 1), higher values were found for the wall material in the absence of oil, suggesting that modified amaranth starch also formed complex aggregates because of its hydrophobic-hydrophilic balance.

3.2.2. Light microscopy

Fig. 6 shows the images obtained by light microscopy of the emulsions at different preparation times. Particles with larger sizes were observed at 30 s of preparation, presenting a heterogeneous distribution that was also confirmed by the DLS results mentioned above. When increasing the preparation time of emulsions (longer than 30 s), a decrease in particle size was detected, as well as a tendency to have a more homogeneous distribution of the emulsion along the image. In the case of 90 s of preparation, samples exhibited a heterogenous particle size along the respective microscopy, having small and big particles, being inappropriate for applications as carrier systems. On the other hand, samples at 270 s of preparation time presented a particle size distribution more homogeneous as compared with shorter preparation times (from 30 to 240 s) and, this tendency was demonstrated with the finding of a unimodal size distribution by DLS (particle size close to 342 nm). A study regarding emulsions based on vitamins D and E with modified starch, as wall material, reported a similar particle size of 311.33 nm for a preparation time of 5 min (300 s) [27]. However, for our system at 300 s, the high shear and stress forces resulted in a rearrangement of the particles, obtaining bimodal distributions of particle size (see Fig. 4 and Table 2). Therefore, it was confirmed that the most adequate preparation time was the sample obtained at 270 s.

3.2.3. Fractal analysis

Fractal dimension (F_D) is a parameter that has been applied to explain morphological changes measured as irregularity of the object's texture. Fig. 7A shows the behaviour of F_D as a function of preparation time of emulsions, where different letters (A, B, C and D) represent means with significant differences ($p \le 0.05$) amongst them. F_D values varied in an interval between 2.74 and 2.85, which indicated an irregular three-dimensional texture of surface for all samples. García-Armenta et al. [36] used this parameter to examine the texture surface of maltodextrin fractured agglomerates and reported no F_D values higher than 2.23. When increasing preparation time from 30 to 60 s, F_D value had a remarkable decrease ($p \le 0.05$). From 60 s of preparation time, F_D values were continuously increasing with an apparently exponential profile towards 210 s. For preparation times higher than 210 s, F_D had no significant changes (p \leq 0.05). These results demonstrated that texture's irregularity increased with preparation time as to reach a maximum irregularity close an F_D value of 2.85. According to literature,

 F_D has been associated with emulsions stability, indicating that more irregular textures (higher F_D values) can trigger a better stability and a good potential as drug carriers [44].

On the other side, Fig. 7B displays lacunarity (L') as a function of preparation time which shows an inverse tendency regarding F_D . As mentioned in the characterisation of modified amaranth starch (see Section 3.1.1), L' is a fractal parameter that indicates the symmetry of the object's surface; therefore, L' was useful to evaluate the distribution of irregularity of particles along the image [36]. The values found for this parameter were between 0 and 0.05; and the more significant variations of L' were observed for samples obtained at 30–210 s. Apparently, the lowest L' value (the highest symmetry) was obtained at 270 s of preparation time and was related to a better distribution of the particles along the image. Consequently, based on DLS results and fractal analysis, 270 s can be considered as the most adequate preparation time for obtaining emulsions based on modified amaranth starch and corn oil.

Besides, a linear regression was performed as a result of the inverse behaviour for both fractal parameters (F_D and L') depending on the preparation time, attaining a good linear correlation ($R^2 = 0.968$) with a negative slope. Therefore, F_D and L' were inversely proportional for this interval of preparation time (30–300 s). This result indicated that this emulsions system tended to have more homogeneous particle size (higher F_D) and well-dispersed particles (lower L') when increasing the preparation time.

3.2.4. Confocal Laser Scanning Microscopy (CLSM)

The emulsion formation at different preparation times was viewed by means of the fluorescence emitted by each component (modified amaranth starch and corn oil) in a confocal laser scanning microscope (Fig. 8). For control samples, emulsions were prepared by using native amaranth starch and corn oil, resulting in an inefficient emulsification as detected by CLSM microscopy, and phase separation was evident since no interfacial region was distinguished. Whereas emulsions from modified amaranth starch and corn oil prepared at 30 s (Fig. 8A) and 270 s (Fig. 8B) clearly exhibited an interfacial region, i.e. the oil phase was successfully coated by the wall material. A reduction of particle size at 270 s was observed for both components (Fig. 8B1 and B2) in comparison to the particles observed at 30 s (Fig. 8A1 and A2). One of the advantages of CLSM is the observation of both wavelength channels merged in one single image (Fig. 8A3 and B3). Therefore, with the use of this tool and the colocalization method, the encapsulation efficiency was estimated around 65% for both preparation times (30 and 270 s). In both cases, this efficiency is reported as an average value that presented less variation for the sample at 270 s. In this regard, the efficiencies of encapsulation obtained in this work were higher as compared with the efficiency of 49.98%, which was reported in a recent study of oil-in-water emulsions prepared by a microfluidization process using gum arabic as wall material [45]. Furthermore, it has been studied that emulsion microstructure has an influence over the encapsulation efficiency given that it promotes the release and viability of the active agent [10,46].

In addition to the remarkable properties of the colloidal system developed in this work, emulsions based on modified starch seem to be promising materials as carriers of host substances. For instance, emulsions involving modified waxy maize starch and corn oil have resulted stable at simulated gastric conditions, observing a higher extent of starch digestion in the small intestine [47]. On the other hand, the starch modification is commonly carried out by using toxic substances and expensive operations. In our case, the modification of the native material was conducted by a standard process, avoiding harmful chemicals and obtaining a high production in a low time with no waste effluents. It is noteworthy that our colloidal system presented suitable and well-distributed particle size (nanometric scale), having a lower mean diameter in comparison to other emulsions based on modified starch (micrometric scale) [48]. Besides, these particles prepared from





60 s



90 s



210 s

240 s



Fig. 6. Light microscopy images of emulsions at different preparation times at 40X magnification. The scale bar represents 10 μ m.



Fig. 7. Morphometric parameters: A) Fractal dimension (F_D) and B) Lacunarity (L') of emulsions images at different preparation times. Different letters (A, B, C and D) represent means with significant differences by a Tukey test ($p \le 0.05$).



Fig. 8. CLSM images of emulsions at 30 s (A) and 270 s (B) of preparation time. Red colour is corn oil in A1 and B1; blue colour is modified amaranth starch in A2 and B2; A3 and B3 are merged images. The scale bar represents 20 μ m (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

renewable materials should be biocompatible and eco-friendly, after a probable excretion from human body, due to their chemical composition

4. Conclusions

The standardisation of preparation methods is an important task to provide improved materials to pharmaceutical and food industries. Thus, in this study, a strategy developed for the isolation of starch from amaranth seeds was satisfactorily applied. After that, the wall material with tailored properties was obtained and proved in emulsions formation using commercial corn oil as oil phase model. The chemical modification of amaranth starch involving crosslinking via reactive extrusion was successfully performed, being that confirmed from results of TGA analysis, DLS measurements and fractals. The modified amaranth starch obtained from 80 s of outlet time from the extruder was the wall material with optimal properties. Similarly, an optimal preparation time of emulsions was observed at 270 s. At this preparation time, results of a thoroughly data analysis and processing from DLS and microscopy converged and indicated that a well-dispersed and stable emulsion system having a suitable particle size and a good encapsulation efficiency (65%) was designed. The most remarkable properties of the modified starch obtained were: (i) high Tg of the wall material, which is associated to a better distribution of the polymeric chains at the interfacial region of the droplets; (ii) tailored hydrophobic-hydrophilic balance resulting in controllable shape of particles having a size lower than 350 nm; (iii) conventional toxic surfactants were not required; (iv) the eco-friendly and biocompatible nature of the system. Thus, the designed wall material was suitable for the proper preparation of surfactant-free emulsions that may act as good carrier of food and drug agents.

CRediT authorship contribution statement

Evangelina García-Armenta: Conceptualization, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. Lorenzo A. Picos-Corrales: Formal analysis, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision. Gustavo F. Gutiérrez-López: Resources, Writing - review & editing. Roberto Gutiérrez-Dorado: Resources, Writing - review & editing. Janitzio X.K. Perales-Sánchez: Methodology, Validation, Investigation. Santiago García-Pinilla: Methodology, Validation. Fernanda Reynoso-García: Methodology, Software, Investigation, Data curation. J. Martín Martínez-Audelo: Methodology, Software, Investigation, Data curation. M. Alejandro Armenta-Manjarrez: Methodology, Software, Investigation, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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