STABILIZING OIL-IN-WATER EMULSIONS WITH YAM BEAN (*Pachyrhizus erosus* L. URBAN) SOLIDS

ESTABILIZACIÓN DE EMULSIONES ACEITE-EN-AGUA CON SÓLIDOS DE JÍCAMA (*Pachyrhizus erosus* L. URBAN)

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Abstract

Yam bean (*Pachyrhizus erosus* L. Urban) solids (YS) were used at concentrations of 3, 5 and 7 % w/w for stabilizing oil-in-water emulsions with a 0.15 mass fraction (E_3, E_5 and E_7). Higher YS concentrations produced emulsions with smaller average particle size distribution, more marked shear thinning behaviour, and higher solid-like (tan δ < 1) viscoelastic properties. Optical micrographs revealed that the rheological properties, particle size distribution, and stability of the emulsions were predominantly associated to the formation of 3-D particles network in the continuous phase that immobilized the emulsion droplets. The micrographs also showed small sized YS fragments adsorbed at the oil-water interface, contributing with a steric stabilization term to emulsions stability. Variations in the rheological properties of the emulsions with aging time (14 days) were less pronounced when using higher YS concentrations.

Keywords: yam bean solids, solids network formation, oil-in-water emulsions, stability, rheology.

Resumen

Se usaron sólidos (YS) de jícama (*Pachyrhizus erosus* L. Urban) en concentraciones de 3, 5 y 7 % p/p para estabilizar emulsiones aceite-en-agua con una fracción másica de 0.15 (E_3, E_5 y E_7). Mayores concentraciones de YS produjeron emulsiones con tamaño de distribución de partícula menor, un comportamiento reoadelgazante más pronunciado, y propiedades viscoelásticas sólidas mayores (tan δ < 1). Micrografías ópticas develaron que las propiedades reológicas, el tamaño de distribución de partícula y la estabilidad de las emulsiones estuvieron predominantemente asociadas a la formación de una estructura entrelazada 3-D de las partículas en la fase continua que inmovilizaron las gotas de la emulsión. Las micrografías también mostraron la adsorción de pequeños fragmentos de YS en la interfase aceite-agua, que contribuyeron a estabilizar la emulsión con un potencial de repulsión de origen estérico. Las variaciones en las propiedades reológicas respecto al tiempo de almacenamiento (14 días) fueron menores al usarse concentraciones de YS mayores.

Palabras clave: sólidos de jícama, formación de una estructura entrelazada de sólidos, emulsiones aceite-en-agua, estabilidad, reología.

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1 Introduction

There is a leading trend towards the use of renewable substrates that have potential applications in the food, cosmetic and pharmaceutical industries with emulsifying, foaming, texturizing, microencapsulation, rheology modifying properties, among others. Interest in them has also been encouraged because of their ready availability, environmental friendly nature, and biodegradability (Colin *et al.*, 2013; Makkar *et al.*, 2011). Frequently the required technology for obtaining products from them is quite simple and inexpensive, and this allows for the development of processing facilities and products that help boost the economy of regional economies (Rayner *et al.*, 2012; Winuprasith and Suphantharika, 2013). When these systems are oriented to be used in products for direct human consumption, they must be prepared entirely from food-grade ingredients using economic and reliable processing conditions. One of the promising routes for producing food-grade colloidal particulates is to depart from raw materials that are legally allowed in foods. Most often than not, colloidal particulate systems obtained from agro renewable substrates, are complex systems containing various kinds of ingredients (protein, starch, cellulose, hemi-cellulose, fat, pectins, etc.). Their fabrication may give rise to a wide range of different sizes (from nano to micron sized), shapes (spherical, non-spherical, fibres, or clusters), and internal structures. The physicochemical characteristics of these particulate systems will determine the way that other molecular species interact with them, and determine the bulk physicochemical properties of the overall system such as rheological properties, texture and stability (Jones and McClements, 2010; Kragel and Derkatch, 2010).

There are several mechanisms by which solid particles may stabilize emulsions, among them: (i) accumulation of particles at the oil-water interface in the form of a densely packed monolayer, that prevents droplet flocculation and coalescence by a steric mechanism (pickering stabilization); (ii) immobilization of droplets in an aggregated 3-D particle network (network stabilization); and (iii) pickering/network stabilization (Nadine *et al.*, 2014). Furthermore, when mixed emulsifier or protein-particle systems are employed, competitive adsorption may occur, but the precise role of one particular entity in formulations is difficult to ascertain in the presence of the other adsorbing species because of the complex interactions between different species present in the system (Murray *et al.*, 2011). Emulsions stabilized with solids are attractive formulations because they are simple and bear strong similarities with the well-known surfactant-based emulsions (Chevalier and Bolzinger, 2013). Systematic studies of stability and rheology of oil-in-water emulsion systems made with particles as sole emulsifiers/stabilizers should provide insight into the relation between adsorbed layer properties and bulk emulsion stability, and to the mechanisms taking place in the conformation and evolution of structures in the vicinity of the interface and in the continuous phase bulk (Fouconnier *et al.*, 2012; Golemanov *et al.*, 2012; Kragel and Derkatch, 2010).

Among the Neotropical legume genera with edible tuberous roots, the yam bean (*Pachyrhizus erosus* L. Urban), is the only one that is extensively cultivated on a large scale, but it is still a largely under-exploited crop plant. Compositional analysis reveals that tuberous root contains starch, pectic polysaccharides, celluloses, hemi-celluloses, and proteins. The relative amount of these components depends on the maturity of the tuberous root (Ramos-de-la-Peña *et al.*, 2013). Yam bean starch has been used for obtaining food-grade films (Mali *et al.*, 2004), as a thickening and gelling agent in food (N'da Kouamé *et al.*, 2011), and for partially substituting refined wheat flour for making bread (Nindjin *et al.*, 2011); Acid thinned starch as fat replacer in stirred yogurt (Amaya-Llano *et al.*, 2008); and the soluble dietary fibre for enhancing the syneresis, microstructure and rheological properties of stirred yogurt (Ramirez-Santiago *et al.*, 2010). Overall, different reports have shown the viability of yam starch as an additive for food products. However, the use of yam starch implies an extraction step with an important increase of processing costs. Recent studies indicate that flour from different botanical sources can perform similarly as their starch counterpart, e.g., rice flour films displayed similar mechanical properties than rice starch based films (Dias *et al.*, 2010). Given the richer composition of rice flour, it offers a greater flexibility under heat-moisture treatment than rice starch (Puncha-Arnon and Uttapap, 2013). Thus, an ongoing research topic is to evaluate the ability of inexpensive flours from different sources as food-grade additives, and to gain insights of their performance in specific food applications. To our knowledge, there are no reports dealing with the use of yam bean solids as such.

The aim of this work was to evaluate the effect of different yam bean solids (YS) concentrations on the

morphology, particle size distribution and rheological properties of oil-in-water emulsions.

2 Materials and methods

2.1 Materials

Yam bean (*P. erosus* L. Urban) tubers were obtained from a local market in Yautepec, State of Morelos, Mexico. Commercial canola oil (CO; Alimentos Capullo S., de R.L. de C.V. Mexico) was purchased in a supermarket. Ethanol (EtOH) reagent grade was purchased from J.T. Baker (Naucalpan, State of Mexico, Mexico). The water used in all of the experiments was deionised.

2.2 Yam solids preparation

The skin of yam bean tubers was removed with a manual peeler. The skinless tubers were then diced and put into a food processor (Moulinex model AR68, Tlalnepantla, State of Mexico, Mexico). The ground particles were washed with lukewarm water and the superficial water was removed with the help of an absorbent paper. Batches (1.5 kg) of moist particles were dried at 48 ± 1 °C with an oven dryer (Felisa mod. 62282; Mexico City, Mexico) until constant weight was achieved ($\sim$ 3 h). The dried particles were ground in a dry mill (Samap Ecosysteme SARL, model F 100, Andolsheim, France) and the resulting dried powders were sieved through US 365 mesh ($<$ 44 μm). The yam solids (YS) passing through the mesh were used in the experiments. The YS powders were analyzed by standard AOAC (1995) procedures, unless otherwise stated: protein (N $\times$ 6.25) by the micro-Kjeldahl; moisture by oven drying; ash content by incineration; and total carbohydrate and starch by the Anthrone method (Morse, 1947).

2.3 Scanning electron microscopy of yam solids

YS samples were fixed on carbon supports using double-side adhesive tape. SEM observations were done with a Jeol 7600F microscope operating at 1 KV under vacuum (9.65×10^{-5} Pa). Different image magnification was used for a visual evaluation of the morphology of the YS.

2.4 Emulsion preparation

Aqueous dispersions of 3%, 5%, and 7% w/w YS were prepared by mixing thoroughly the blends. The dispersions were let to stand for 24 h to allow complete hydration. Oil-in-water emulsions with a dispersed mass fraction (ϕ) of 0.15 were prepared by adding dropwise the requisite amount of CO to the YS dispersions with the help of a Silverson L4R high shear homogenizer (Silverson Machines, Ltd., Waterside, Chesham, Buckinghamshire, England) operated at 10 000 rpm during 10 min. The resulting emulsions were coded E_3, E_5, and E_7.

2.5 Light microscopy

Light microscopy was used to examine the morphology of the emulsion droplets and of YS aggregates in the continuous phase. For improving image contrast, the YS were tinged with methylene blue. As the concentration and size of the YS and of the oil droplets in the emulsion were quite dissimilar, viewing of the emulsions was carried out in two fashions: (a) A single drop of undiluted emulsion was put on viewing slides, upon which coverslips were gently placed. This allowed appreciation the 3-D structures and assorted debris formed by YS in the continuous phase at magnifications of 10 x; and, (b) 1 mL of emulsion diluted in 100 mL of water and observed at a magnification of 100 x. This permitted the observation of oil droplets in sufficient detail for appreciating the presence of adsorbed material at their surface and of material lying in the periphery of the droplets. An Olympus BX45 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) was used and images were captured with Olympus E-620 digital camera.

2.6 Zeta potential measurements

The zeta potential of E_3, E_5 and E_7 freshly made, CO and YS dispersion (0.5 % w/w), was measured with a zeta potential analyser (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK). The instrument provides measures of the direction and velocity of a particle in an applied electrical field by means of phase analysis light scattering and laser Doppler velocimetry. The estimated electrophoretic mobility is converted into zeta potential values using the Smoluchowski model.

2.7 Particle size distribution

The particle size distribution (PSD) of the YS (3, 5 and 7 % w/w) dispersions aged 24 h and of the E_3, E_5 and E_7 aged 1 h and after 14 days storage was measured by static light scattering using a Mastersizer 2000 particle size analyser (Malvern Instruments Ltd., Malvern, UK). The refractive indexes used were 1.342 for the emulsions samples and 1.33 for deionised water (dispersant medium).

2.8 Rheological measurements

A Physica MCR 300 modular compact rheometer (Anton Paar Messtechnik, Stuttgart, Germany) with truncated cone-plate geometry (2°, 50 mm diameter, gap of 0.05 mm) was used for estimating the rheological properties of the emulsions. To this end, the emulsion samples were carefully placed in the measuring system, left to rest for 15 min for structure recovery and temperature equilibration at 25 °C. The apparent viscosity (η_{app}) versus shear rate ($\dot{\gamma}$) behaviour of the samples was determined by applying an increasing shear rate from 0.01 to 1000 s^{-1}. Amplitude sweeps were carried out to characterize the viscoelastic linear region (LVR) of the samples, with strain ranging from 0.01 to 100% at 1 Hz, followed by frequency sweep from 0.01 to 1 Hz at 0.5% strain (corresponding to the LVR) (Dzul-Cauich *et al.*, 2013). The loss factor tan δ = G"/G' was obtained from the equipment software. Measurements were performed on the emulsions after 1 h and 14 days of storage, and at 25 °C.

2.9 Statistical analyses

Data were analyzed using a one-way analysis of variance (ANOVA) and a Tukey's test for a statistical significance P ≤ 0.05, using the SPSS Statistics 19.0 (IBM Corporation, New York, USA). All experiments were done in triplicate.

3 Results and discussion

3.1 Morphology of yam solids

Chemical composition analysis of yam bean tubers similar as those used in this work indicates that they possess a high level of moisture (> 80%), appreciable amounts of carbohydrate (about 15%), crude fibre (about 1.5%) and protein (< 1.3%) and negligibly low amount of lipid (< 0.1%) (Bhandari *et al.*, 2003).
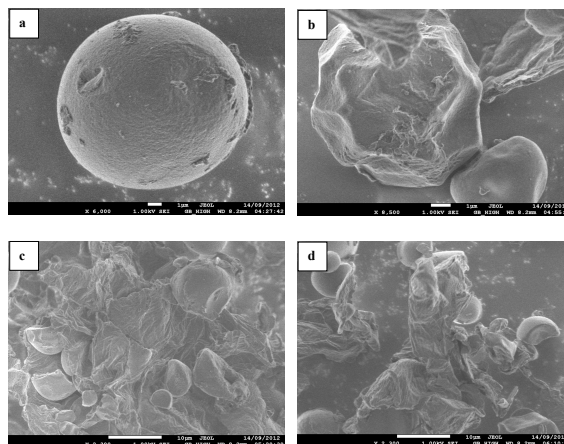

Fig. 1. SEM images of yam solids morphologies, showing a diversity of sizes and shapes: Intact spherical (a) and polygonal (b) particles; and an array of amorphous structures (c) and solids fragments (d).

The proximate chemical composition of YS powders obtained in this work was as follows (per 100 g powder): 6.8 ± 0.5 g of H_2O (93.2 total solids); 9.1 ± 0.8 g of protein; 3.4 ± 0.3 g of ash; 76.1 ± 6.3 g of carbohydrate (of which 66.5 ± 7.1 g were of starch).

This complex composition is reflected in the morphology of the YS shown in Fig. 1, where and heterogeneous diversity of particles sizes and shapes can be observed. Fig. 1.a shows a spherical starch granule with a rather smooth surface upon which some smaller fragments are imbedded, while Fig. 1.b. shows a polygonal starch granule showing quite rough surface and edges, partially coalescence with smaller starch granules fragments. Figs. 1.c and 1.d show that the bulk of particles are characterized by a heterogeneous array of sizes and shapes, resulting from the fragmentation of yam tuber tissue when subjected to grinding-drying process. These fragments could correspond to any of the components mentioned above. These results are in agreement with those by Stevenson *et al.* (2007) who reported that starch granules of yam harvested in Texas and Mexico had spherical or polygonal morphology of varying sizes.

3.2 Zeta potential

Given the diversity sizes, shapes, and source of the YS components, it is possible that some of them possess ionisable functional groups that induce electrostatic charges. Fig. 2 shows that YS (0.5 % w/w) dispersion in water exhibited an effective negatively charge of -27.9 mV, while canola oil was practically electrostatically neutral (0.1 mV).
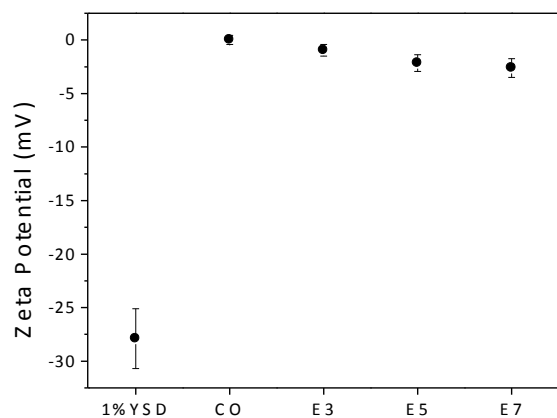
Fig. 2. Zeta potential of emulsions made with different yam solids concentration, of 1 % w/w yam solids dispersion, and of canola oil.

The zeta potential value displayed by the YS dispersion was within range of that reported for starch dispersions from different botanical origin (Chanamai and McClements, 2002). This result is of importance because particle-particle interactions (in this case repulsive) should have bearing on the stability and rheological properties of the oil-in-water emulsions. The zeta potential displayed by the E_3, E_5 and E_7 is also shown in Fig. 2. It is observed that the zeta potential of the emulsions was considerably less than that of the 0.5% w/w YS dispersion, suggesting that as the YS concentration was increased, the ionization of functional groups was hindered and shielded due to crowding effects among molecules and increased presence of counterions in the neighbourhood of the ionisable groups. Nevertheless, the zeta potential of the emulsions (Fig. 2) was slightly negative increasing from about -1.0 mV to an asymptotic value of about -2.2 mV as YS concentration increased from 3% to 7% w/w. This phenomenon has been observed for the adsorption of synthetic polyelectrolytes where the final surface tends to achieve a maximum value independently of concentration (Schoeler *et al.*, 2003; Zinoviadou *et al.*, 2012).

3.3 Emulsion morphology

Fig. 3 shows the light microscopy micrographs of the undiluted E_3, E_5 and E_7. Inspection of the micrographs reveals that all the emulsions showed a continuous phase characterised by an array of differently sized and shaped YS particles (reflecting that observed in Fig. 1), forming 3-D aggregated structures, where much smaller interspersed oil droplets were
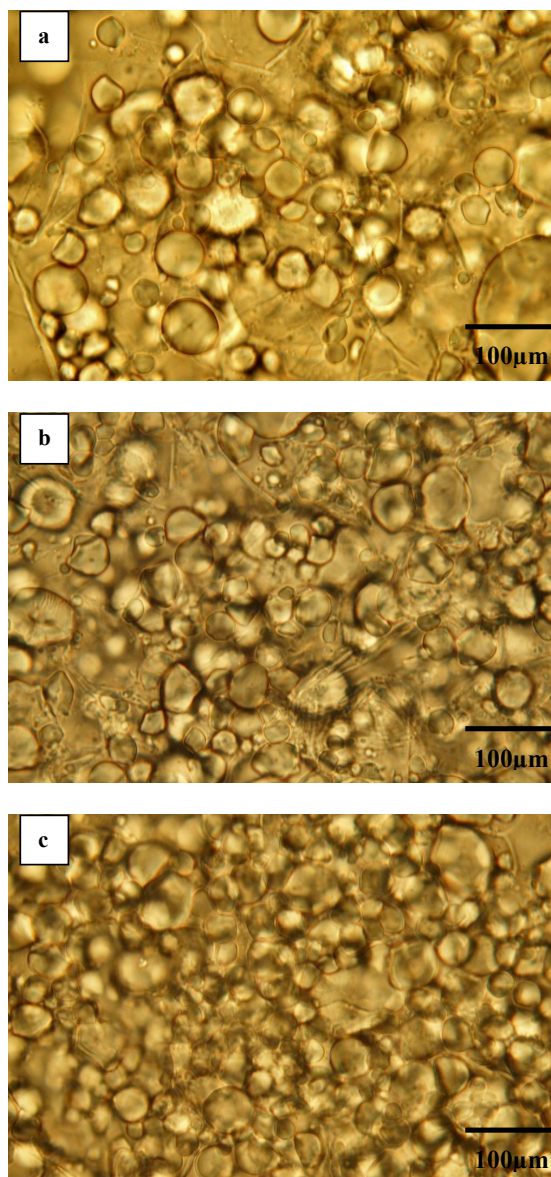

Fig. 3. Light micrographs of the undiluted emulsions made with different yam solids concentration: (a) 3 % w/w; (b) 5 % w/w; and (c) 7 % w/w.

observed. Although it is difficult to discern from the micrographs if solid particles were adsorbed on the droplets surfaces, the presence of some irregular droplet shapes indicates that this may be the case. Pickering emulsions tend to exhibit irregular shapes due to the non-uniform wetting of surface-active particles in the interface (Vignati *et al.*, 2003).

Comparison of Figs. 3 a, b and c, allowed to establish that as the concentration of YS increased in the emulsions, two phenomena took place: (i) an
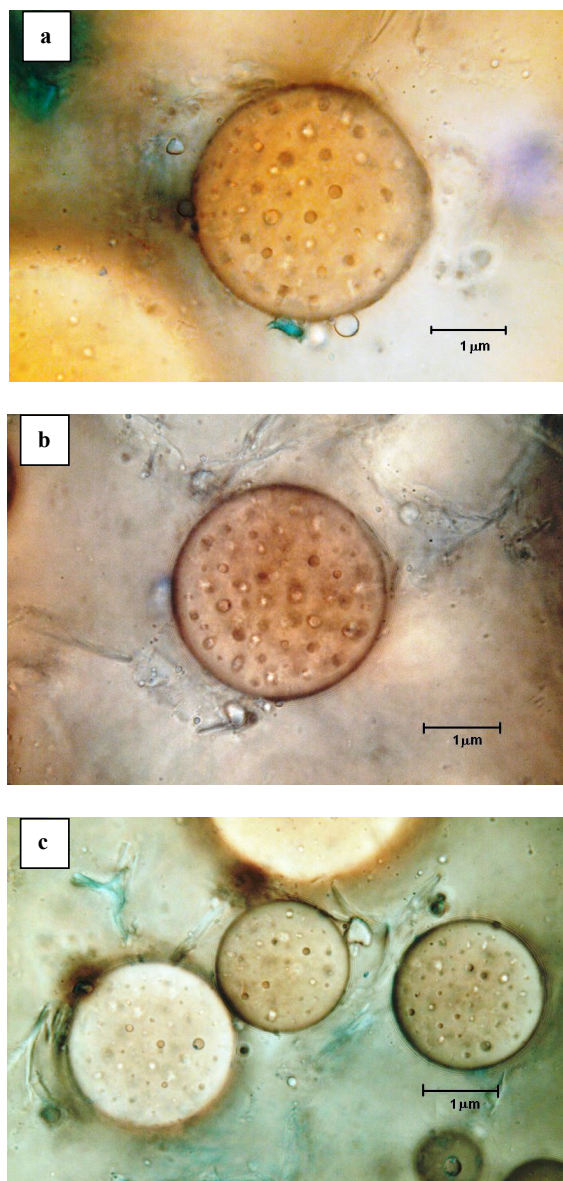
Fig. 4. Light micrographs of the diluted emulsions made with different yam solids concentration: (a) 3 % w/w; (b) 5 % w/w; and (c) 7 % w/w.

increasing higher number of irregularly shaped entities occurred, and (ii) the size of the dispersed components in the continuous phase tended to diminish and to be more uniform. The size reduction could have occurred due to the attrition between the densely packed components during the shearing process, causing the formation of larger flocculated solid particles network in the continuous phase that hindered the mobility of individual oil droplets.

Figs. 4.a and 4.b show micrographs of the diluted

emulsions viewed at a higher magnification. Both micrographs show single emulsion oil droplets, in which it can be appreciated that relatively small solid particles and filament-like entities (see Figs. 1 c and d) were adsorbed upon or lying nearby the oil droplet surface. Frontal viewing of the droplets shows different sized crater-like indentations presumably due to solids adsorbed on the surface. Notice that surface coverage by the solids is far from being complete. In Figure 4.c, bridging between droplets by filamentous strands and droplets sharing an adsorbed solid can be appreciated. Thus, we may infer from the structures visualized in Figs. 3 and 4, that a rather complex system is formed. On one hand, small sized particles were adsorbed sparingly at the oil droplets surfaces, while the large sized particles formed an aggregated structure, which conferred stability by immobilizing the oil droplets (Dickinson, 2012).

3.4 Emulsion particle distribution and stability

Images in Fig. 3 indicate that the continuous phase was made up by a complex entanglement of YS forming a 3-D network system, where smaller oil droplets were embedded within it. This structure can mask the determination (via Mastersizer 2000 particle size analyzer) of the actual emulsions oil droplet size distribution. Thus, in a first instance, the particle size distribution the YS dispersions (3%, 5% and 7% w/w) was determined. The PSD of all the YS dispersions (Fig. 5) was very wide with particle sizes ranging in between 0.5 to 500 μm. The PSD was characterised by a peak at around 115 μm, and three shoulders (two to the left and one to the right). The smallest shoulder occurred at around a mean particle diameter of $\sim$ 1.5 μm, followed by one at $\sim$ 13 μm. The shoulder to the extreme right corresponded to $\sim$ 230 μm. These results confirm that YS are made up by a heterogeneous array of particles. It is interesting to notice that PSD was practically insensitive to YS concentration in the dispersions.

The PSD of the fresh emulsions (Fig. 6.a) had well defined peaks compared to the PSD of the YS dispersions at different concentrations (Fig. 5), indicative that probably the shearing process had a homogenising effect on particles sizes. The peak occurring at $\sim$ 1.2 μm may be mainly attributed to the formation of oil droplets (Fig. 4), while the peaks at higher sizes were mainly due YS (Fig. 3).
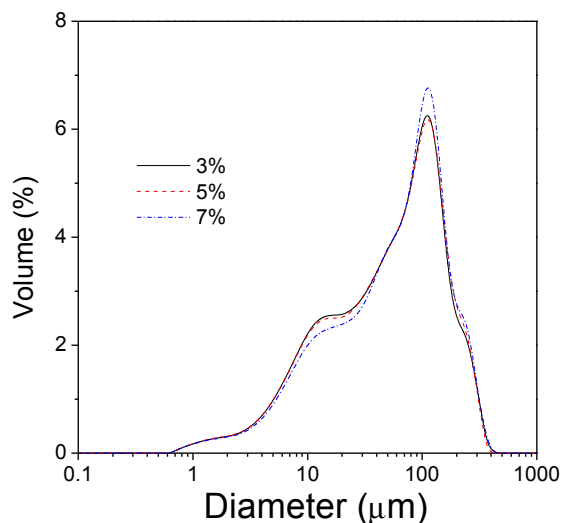
Fig. 5. Particle size distribution of yam solids dispersions at different concentrations.
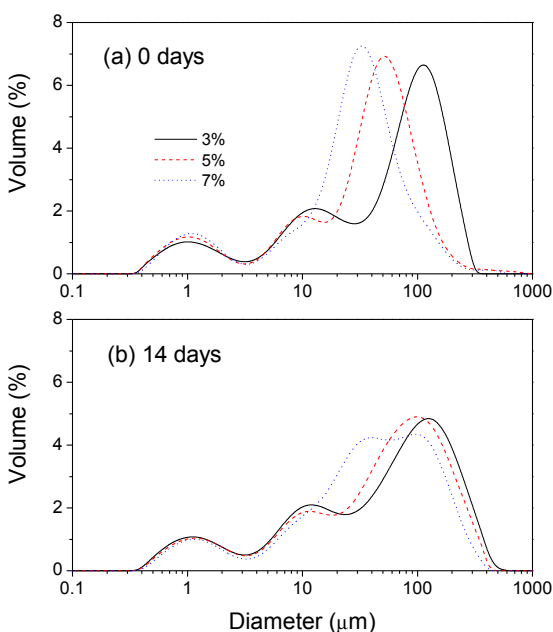

Fig. 6. Particle size distribution of the emulsions made with different yam solids concentrations: (a) freshly prepared; and (b) aged 14 days.

The Gaussian deconvolution of the particle size distribution indicated that the area of the left most peak was about 12-19 %, which was in the range of the oil phase disperse fraction (0.15). The peaks corresponding to the higher sized particles, tended to be shifted to the left, the higher was the YS concentration, probably because of the higher

degree of attrition incurred by the particles under harsh shearing stresses. In contrast, the position peak corresponding to ~ 1.2 μm, was unaffected by YS concentration. This fact seems to indicate that oil droplet size distribution was not affected by YS concentration in the dispersion, and that the YS particles adsorbed at the interface did not decrease interfacial tension. These results are in line with the light microscopy micrographs shown in Figs. 3 and 4.

Fig. 6.b presents the PSD of the emulsions aged 14 days. For E_3 and E_5 both peaks to the left remained almost the same with respect to those observed for the fresh emulsions; in contrast, the peak to the extreme right, corresponding to the large size particles distributions, shifted towards larger sizes with aging time. In the case of E_7, the single peak corresponding to large sizes deconvoluted into a rather broad flat peak. These results suggest that the small sized particles maintained their size with aging time, corroborating that they were maintained immobilised in the 3-D network structure formed by the larger YS particles. On the other hand, the very large YS tended to aggregate into of larger flocs with aging time. These results confirm that emulsions made with polydisperse, non-spherical aggregates, can be conferred impressive emulsion stabilisation properties by a combination a steric repulsive term caused by the adsorption of solid particles at the oil droplets surface, but mainly through the immobilisation of the oil droplets in a gel-like particle network (Dickinson, 2010). Electrostatic contributions to stability in this scheme are negligible.

3.5 Emulsions rheology

The steady shear viscosity of the fresh emulsions and aged 14 days is presented in Fig. 7. All of the emulsions presented typical structural viscosity behaviour characterized by presenting a upper Newtonian viscosity plateau at low shear rates (η_0), a shear shear-thinning region at intermediate shear rates, and lower Newtonian viscosity plateau a high shear rates (η_∞). This type of behaviour is characteristic of materials that when put to rest after shearing show time-dependent restructuring. In all of the emulsions this structural behaviour was shown independently of YS concentration and of emulsions aging time. The $\eta_{app} - \dot{\gamma}$ flow curves were slightly higher for the fresh emulsions than for those aged 14 days, particularly at relatively low shear rates. As YS concentration decreased, the decrease in emulsions η_{app} was larger with aging time.
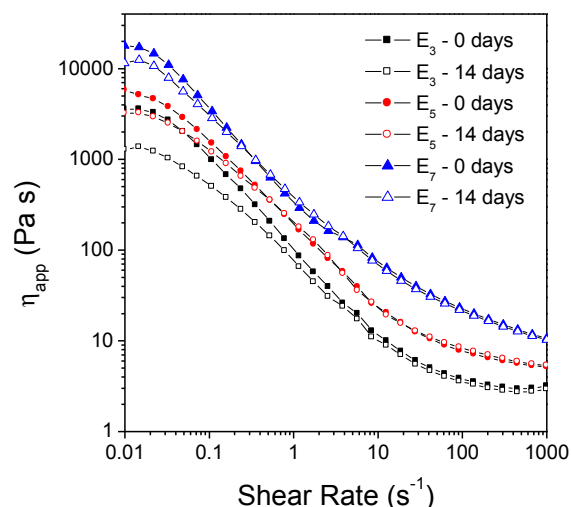
Fig. 7. Apparent viscosity of the fresh emulsions and after 14 days of storage time. A shear-thinning behaviour is noted for all YS fraction, and this behaviour is also exhibited after 14 days of storage time.
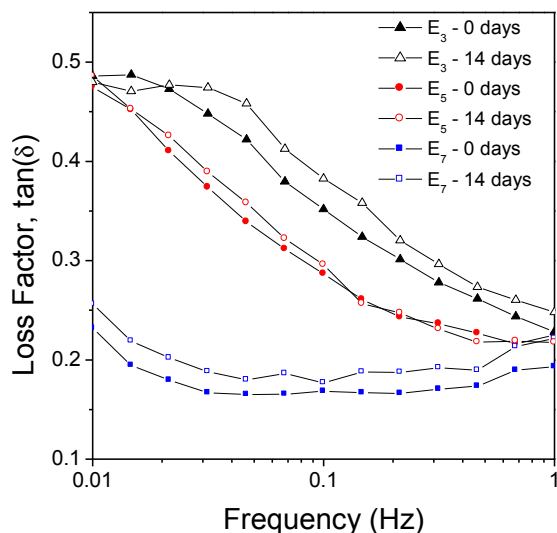

Fig. 8. Loss factor tan(δ)=G''/G' for emulsion for the three YS concentrations. Elasticity effects dominate over viscous ones, conferring a gel-like behaviour to the emulsions.

In concentrated mixed suspensions containing droplets and solids, the flocs are close enough together to interact with each other, through hydrodynamic interactions, colloidal interactions, or entanglement (McClements, 2005). At sufficiently high droplet and solids concentrations, flocculation may lead to the formation of a 3-D network of aggregated droplets

and solids. Network formation causes a suspension of particles to exhibit viscoelastic characteristics (Pal, 1996). The network of aggregated droplets acts like a solid at low shear stresses because the applied forces are not sufficient to overcome the forces holding the particles together, exhibiting a constant high viscosity. As a critical shear stress is exceeded, the bonds between particles are disrupted and they can flow past one another. At this point the suspension exhibits strong shear thinning characteristics. At very high shear stresses the 3-D structure is completely broken and a region of constant low viscosity is reached (Sherman, 1968). When the disperse phase volume fraction is higher, the packing of the flocs is more closed and apparent viscosity changes are less noticeable than when the disperse phase volume fraction is lower (McClements, 2005).

Dynamic frequency sweep tests were also performed for determining the frequency dependence of the storage modulus (G') and the loss modulus (G''). Both G' and G'' increased with increasing frequency (data not shown), but for all YS concentrations the fresh emulsions presented a predominantly solid-like behaviour since tan δ = G''/G' < 1 over the entire frequency range examined (Fig. 8). Similar behaviour has been previously observed for emulsions that were stabilized by solids where a 3-D network provided long-term stability to the dispersed oil drops and a solid-like textural character to the whole emulsion (Dickinson, 2010). In this way, it is suggested that while steric hindrance by active YS adsorption may contribute to the stability and rheological behaviour, it is the highly structured non-active YS 3-D network in the continuous phase that controls the rheology and stability of the emulsions (Lorenzo *et al.*, 2013). After 14 days, the rheology of the emulsions was still dominated by a solid-like behaviour, although this dominance decreased, possibly due to the appearance of discontinuous patches of flocs in the continuous phase.

Conclusions

In this work the ability of yam bean (*Pachyrhizus erosus* L. Urban) solids for stabilizing emulsions was explored. Yam solids were characterized by a complex composition that mainly comprised protein and carbohydrates (predominantly starch). Scanning electron micrographs showed the presence of spherical and polygonal starch granules, and an array of smaller fragments resulting from the fragmentation of yam

solids during their processing. In this way, some small yam solids adsorbed at the interface contributing to emulsion stability, but non-adsorbing yam solids formed densely packed 3-D aggregated structures in the continuous phase, that entrapped emulsion droplets and hindered their mobility. Higher yam solids concentrations resulted in emulsions with enhanced flow and viscoelastic properties, and with reduced variation in the rheological properties with aging time. This study opens up a new route for easily preparing and obtaining inexpensive food grade solids for stabilising emulsions with predominantly gel-like behaviour, which may on turn be used for structuring more complex functional foods.

Acknowledgment

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