CARACTERÍSTICAS MICROMORFOMÉTRICAS DE EMULSIONES DE α-TOCOFEROL OBTENIDAS POR MICROFLUIDIZACIÓN

MICROMORPHOMETRIC CHARACTERISTICS OF α-TOCOPHEROL EMULSIONS OBTAINED BY MICROFLUIDIZATION

C. Cano-Sarmiento[1], A. Monroy-Villagrana[1], L. Alamilla-Beltrán[1], H. Hernández-Sánchez[1], M. Cornejo-Mazón[2], D.I. Téllez-Medina[1], C. Jiménez-Martínez[1] and G.F. Gutiérrez-López[1*]

[1]*Departamento de Graduados e Investigación en Alimentos, Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional. Carpio y Plan de Ayala s/n. Col. Santo Tomas. México, D. F. CP. 11340*
[2]*Departamento de Biofísica, Escuela Nacional de Ciencias Biológicas-IPN.*

Abstract

This work aimed to study the effect of different types of surfactants (non-ionic, cationic, anionic) on the micelle size and aggregation pattern of α-tocopherol emulsions produced by microfluidization prepared by using one and two cycles. Tween-20, Lecithin (PhC) and dodecyl sodium sulfate (SDS) were used as the non-ionic, cationic and anionic surfactants, respectively. Particle size was determined by image analysis and agglomeration was characterized with micromorphometric parameters such as fractal dimension (FD) and lacunarity (Λ). Average size was maximum (10.65 μm) when using Tween-20, whereas this parameter was the lowest (2.76 μm) when using PhC. The greater contributions to changes in FD and Λ were due to the presence of Tw and SDS. With PhC, it was possible to observe a system with high values of FD (1.92) and low values of Λ (0.15). PhC contributes to the stability of the emulsion despite of the concentration and number of microfluidization cycles and presented better dispersion and more irregular micelle structures than when using other surfactants.

Keywords: agglomerates, surfactant, fractal dimension, lacunarity and microfluidization.

Resumen

En este trabajo se estudió el efecto de diferentes tipos de surfactantes (no iónico, catiónico, aniónico) en el tamaño y forma de las micelas de emulsiones de α-tocoferol (AT) producidas por microfluidización usando uno y dos ciclos. Tween-20 (Tw), lecitina (PhC) y dodecil sulfato de sodio (SDS) se utilizaron como surfactante no iónico, catiónico y aniónico, respectivamente. El tamaño de partícula se determinó por análisis de imágenes y la presencia de aglomeración se caracterizó a través de parámetros micromorfométricos como la dimensión fractal (FD) y lagunaridad (Λ). Se alcanzó el tamaño máximo (10.65 μm) de micela cuando se utilizó Tw, mientras que este parámetro fue el más bajo (2.76 μm) cuando se utilizó PhC. Se encontró que las mayores contribuciones a los cambios en la FD y Λ son debido a la presencia de Tw y SDS. Con la PhC se pudo observar un sistema con valores altos de FD (1.92) y valores bajos de Λ (0.15). La PhC contribuyó a la estabilidad de la emulsión indepedientemente del número de ciclos de microfluidización y de su concentración y presentó una mejor dispersión y estructuras micelares más irregulares que con el uso de los otros surfactantes.

Palabras clave: aglomerados, surfactante, dimensión fractal, lagunaridad y microfluidización.

Corresponding author. E-mail: gusfgl@gmail.com

1 Introduction

Vitamin E is an essential micronutrient in human and animals diets due to its biological activity and its powerful antioxidant activity. Vitamin E refers to a class of molecules that are related in their chemical structures and biological activities among which the most important is AT (Yang and McClements, 2013a), which is a powerful antioxidant present in biological and food systems (Hoppe and Krennrich, 2000; Ricciarelli *et al.*, 2002; Eitenmiller and Lee, 2004; Zingg and Azzi, 2004; Schneider, 2005; Sagalowicz and Leser, 2010; Fujita *et al.*, 2012; Borel *et al.*, 2013) and there has been a great interest in the fortification of many processed foods and beverages with this compound (Gonnet *et al.*, 2010).

AT can be easily oxidized by the action of heat and light during processing, transport and storage (Bramley, *et al.*, 2000; Gawrysiak-Witukska *et al.*, 2009) thus making incorporation of AT in commercial products a difficult task due to its chemical instability, low water solubility and consequently highly variable bioavailability (Gonnet *et al.*, 2010; Yang and Huffman, 2011; Yang and McClements, 2013b). Its low water solubility implies that it cannot be readily dispersed in aqueous-based products, which has been overcome by protecting AT into a matrix system formed by biopolymers such as gum arabic (GA), maltodextrin (MD), etc. in the form of an emulsion (Chiu and Yang, 1992; Chen and Wagner, 2004; Gonnet *et al.*, 2010, McClements *et al.*, 2009; Feng *et al.*, 2009; Yang and McClements, 2013b; Mayer *et al.*, 2013a).

Physicochemical properties of food emulsions include: size, charge and interfacial properties of individual micelles/micellar agglomerates (M/MA) (Friberg *et al.*, 2004; McClements, 2005; McClements, 2011) and can be manufactured by using a variety of high and low-energy methods. The former include mechanical devices that generate intense breakdown forces such as high-pressure valve homogenizers, microfluidizers and ultrasonic devices which blend oily and aqueous phases and wall materials with or without the addition of surfactants (Jafari *et al.*, 2006; Ciron *et al.*, 2010; McClements, 2012). Low-energy methods are based on the spontaneous formation of oil drops in surfactant-oil-water mixtures (Mayer *et al.*, 2013 a and b; Saberi *et al.*, 2013). The sizes of drops that can be produced depend on the process operating conditions and system composition (Jafari *et al.*, 2007a, 2008; Boom, 2008; McClements and Rao, 2011).

Microfluidization is a high-energy homogenization that induces turbulent mixing, localized energy dissipation which generate a uniform pressure profile (Jafari *et al.*, 2007a). Distribution of the particle sizes produced by a microfluidizer has been reported to be narrower than with traditional homogenization methods (Dalgleish *et al.*, 1996; Perrier-Cornet *et al.*, 2005; Jafari *et al.*, 2006, 2007a) and is the result of competition between breakdown and coalescence and (when used) the effects of emulsifiers which adsorb in the newly formed interface. If collision time between micelles is shorter than adsorption of surfactant, the new interface cannot be completely covered by the emulsifier and coalesce. Therefore, drops coalescence will depend on both, emulsifier adsorption and drop collision rates. Since newly formed drops are not completely covered by emulsifier, a higher collision rate produces a higher coalescence rate (over-processing) which can be prevented through an appropriate selection of emulsifiers and adjustment of their concentration (Jafari *et al.*, 2008). An emulsifier is an active surface molecule which enables drop breakdown and avoids aggregation (McClements and Rao, 2011; Kralova and Sjoblom, 2009).

The nature of drop interactions determines the structure of the newly formed M/MA, their rheology and stability of produced emulsion (Friberg *et al.*, 2004; McClements, 2005). Interaction between drops will be influenced by the components of the emulsion, mainly by those in the interface, which is formed by active surface molecules (surfactants, polymers, proteins, etc.), which in turn interact during the formation of emulsion and provide different features to the dispersed system (Wilde, 2000). During interaction between drops, there are different possibilities of drop-surfactant arrangements, since molecules can compete for adsorption sites, or can mutually promote their adsorption (Petrovic *et al.*, 2010; Chaparro-Mercado *et al.*, 2012).

Digital image analysis (DIA) is a powerful and advantageous tool used in the assessing of structures and can perform evaluation of microstructural properties and evidence subtle differences in aggregates morphology, which may be carried out by fractal analysis (Barleita and Barbosa-Canovas, 1993). The word fractal comes from the latin word *fractus* that means broken, and the corresponding latin verb *frangere* which means breaking (to create irregular fragments) and which was introduced by Mandelbrot (1983), to characterize spatial and temporal phenomena that are continuous (in space and/or time) but not defined.

Fractal theory proposes different methods for describing the inherent irregularity of phenomena or objects (Veleva *et al.*, 2013). This description process is characterized by a parameter known as fractal dimension (FD) (Kenkel and Walker, 1996) which in turn may be estimated by using a number of algorithms which selection depends on available data and pursued information (Lopes and Betrouni, 2009). Fractal dimension of the contour of objects can be determined by applying the box-counting algorithm in which binarized images (2-bits) are separated from their contour and assessed by using boxes of different pixel sizes in a geometric progression (Barleita and Barbosa-Canovas, 1993). Agglomerates with relatively smooth contours have lower fractal dimensions (close to 1) than those with rough borders (close to 2). On the other hand, the lacunarity (Λ) has been used to assessing objects with similar fractal dimensions and which may possess different morphological appearance. Lacunarity gives information about heterogeneity of empty spaces of fractal structures and which are intercepted in different proportion by the box in the above described box counting method (Lobato-Calleros *et al.*, 2009).

Morphological description by using fractal dimension and lacunarity has been applied to systems such as crystals (Velázquez-Camilo *et al.*, 2010), biological tissues (Pantic *et al.*, 2013), yogurt (Lobato-Calleros *et al.*, 2009), blood plasma (Dàvila and Parés, 2007), maltodextrin agglomerates (Meraz-Torrez *et al.*, 2011) and others. Fractal dimension and lacunarity provide complementary information on particle morphology and with heterogeneity or structure of spaces among particles, these parameters can be linked, for example, to physicochemical properties of the assessed system. In this study, we characterized micelle agglomerate, through micromorphometric parameters by using FD and Λ evaluations as a measure of irregularity of M/MA and heterogeneity of their distribution or dispersion in the continuous phase as exemplified in Figure 1. Based in findings from previous studies on the preparation of AT emulsions, (Quintanilla-Carvajal *et al.*, 2011; Omar *et al.*, 2009), the aim of this work was to evaluate the effect of Tween 20, lecithin and dodecyl sodium sulfate (nonionic, cationic, and anionic surfactants respectively) on micelles and micelle agglomerates size and morphology of AT emulsions produced by microfluidization and by using MD and GA as wall materials.
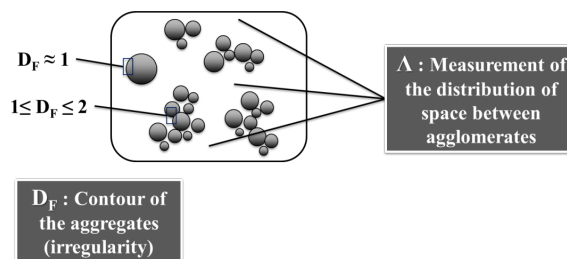

Fig. 1. Representation of fractal dimension and lacunarity in emulsions.

2 Material and methods

2.1 Materials

(±) α-tocoferol (AT) (Sigma Aldrich, Germany), maltodextrin DE20 (MD) (CPI Ingredientes, Mexico), gum Arabic (Norevo, Germany), Tween 20 (Sigma Aldrich, USA), soy lecithin (Sigma Aldrich, USA), dodecyl sodium sulfate (Sigma Aldrich, USA) and type I water (Millipore, Ireland) were used as ingredients of the emulsion.

2.2 Preparation of pre-emulsions

For preparation of pre-emulsions, AT and either Tw, PhC or SDS were slowly added to the solution containing the wall material (gum arabic/maltodextrin) while stirring using a knife blade type homogenizer (Oster, model 2612, USA) during 2 minutes and a blank pre-emulsion was prepared without the addition of surfactant. Solute content of the final pre-emulsion was about 12% and studied formulations are specified in Table 1. Based on preliminary experiments, surfactant concentrations of 0.80 and 1.60 % w/w were chosen for preparing the emulsions. These concentrations fall within the ranges used for preparing emulsions with added surfactants (McClements, 1994). Three pre-emulsions for each surfactant type and three with no surfactant were prepared, for a total of 12 pre-emulsions.

2.3 Microfluidization

For emulsion formation a M-110Y microfluidizer (Microfluidics®, Newton, MA, USA) was used, fitted with two interaction chambers: the primary one of the type "Y" (F20Y, $\phi =75 \mu$m) and the secondary one of the type "Z" (H30Z, $\phi = 200 \mu$m).

Table 1. Formulation of preemulsion and emulsions at 1 and 2 microfluidization cycles

Component	Amount (%(w/w))
α-T	2.83
MD	4
GA	4
Tw, PhC, SDS	0, 0.80 and 1.60
Water type I	89.17, 88.37 and 87.57

The air chamber of the equipment operated at 40 MPa with a liquid pressure of 68 MPa (Microfluidics, 2008) and by passing emulsions 1 and 2 times (cycles) through the equipment. The 12 pre-emulsions described in 2.2 above, were subjected to 1 and 2 cycles of microfluidization.

2.4 Image capture

1 mL samples of the produced emulsions were transferred to imaging immediately after formation of the pre-emulsion or emulsion by microfluidization by using a digital camera coupled to a light microscope (Nikon Digital SightDS-2Mv, TV Lens 0.55XDS, Japan) coupled to a personal computer (2.67 GHz, 1.0 GB RAM) by means of NIS-Elements F2.30 software. Images were acquired at 600x of total amplification with a resolution of 1280 × 960 pixels (3 μm/pixel) and were finally stored in *.jpg format. A Neubauer chamber (Boeco, Germany) was used to establishing the reference observation scale.

2.5 Particle size (PS) and image analysis

Average PS for each emulsion was determined by microscopy trough image analysis (Tansel and Sevimoglu, 2006; Zuñiga *et al.*, 2012; Schuster *et al.*, 2012). 5 fields of view were observed in an optical microscope Nikon H55O5 (Japan) with halogen lighting and maximum diaphragm aperture. Images were processed by means of the ImageJ 1.44 (NIH, USA) software (Abramoff *et al.*, 2004); contrast and gray-scale threshold level (threshold) were adjusted automatically and manually, respectively in order to define individual object contours and thereby producing a binary image. The *Binary: Fill Holes* function of the program, was used to fill out the gaps or holes, and finally the option *Analyze Particles* was applied. Images were saved in *.jpg format, and area, Feret diameter, and shape factor of individual species were obtained.
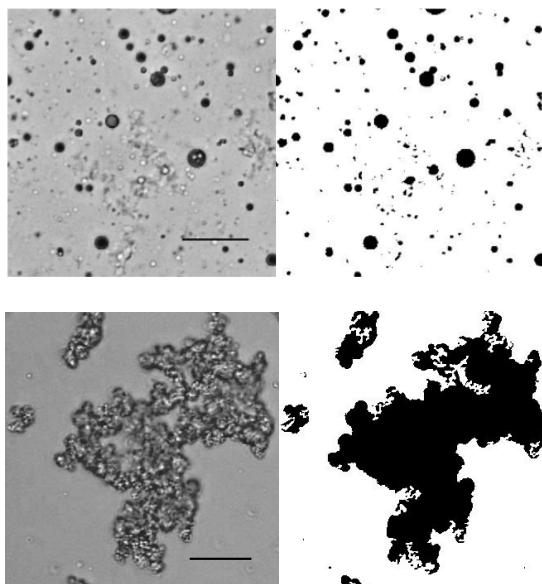

Fig. 2. Example of images taken with optical microscope (gray-scale and binarized). Value of scale is 50 μm in both cases.

Contour fractal dimension (FD) and lacunarity (Λ) of micelles were determined by using the FracLac 2.5 plugin (Karperien, 2004; Dàvila and Parés, 2007; Meraz-Torrez *et al.*, 2011; Pantic *et al.*, 2013) and five crops were obtained from each image for further analysis. In Figure 2, an example of obtained images and processing is presented.

2.6 Statistical analysis

Statistical tests were performed by using the software Minitab 16 (Minitab Inc., USA) and SigmaStat 3.5 (SigmaSoft, USA), which consisted of two-way analysis of variance (Two-way-ANOVA), two-way analysis of variance for repeated measures (Two-way-RMANOVA) and Principal Component Analysis (PCA). The level of significance for each test was established at $2\alpha = 0.05$. When necessary, Student Newman-Keuls test was used for comparison of means (Montgomery, 1991).

3 Results and discussion

3.1 Effect of the concentration surfactants

Examples of images of the pre-emulsion and emulsions prepared by using 0.80% of surfactant are presented in Figure 3.
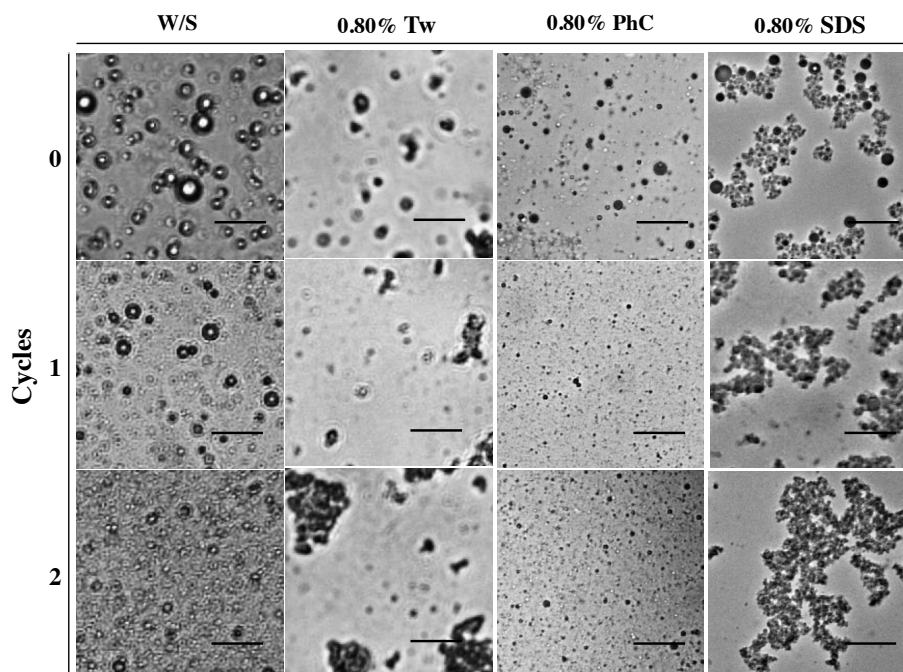
Fig. 3. Images (gray-scale) of prepared emulsions with different surfactants (concentration of 0.80 %) and microfluidization cycles. "0" is pre-emulsion and "W/S" is without surfactant. Length of reference line is 50 μm.

Formation of micellar agglomerates in emulsions prepared by using Tw and SDS can be observed, whereas emulsions without surfactant and containing PhC had a better micelle dispersion.

In Figure 4 (a,b and c), it is possible to observe size, FD and Λ of M/MA of the different emulsions prepared by adding 0.80% of the different surfactants. Sizes (Figure 4a) were statistically different for each surfactant used and maximum (10.65 μm) and minimum (2.76 μm) average sizes were observed for pre-emulsions and microfluidized emulsions prepared by adding Tw and PhC respectively. In emulsion without surfactant and with PhC microfluidization decreased the mean diameter of emulsions; however when using Tw and SDS (nonionic and anionic surfactants respectively) microfluidization did not affect the size and induced micellar agglomerate formation which was related to interactions between both surfactants with the biopolymers due to the competition for interface and displacement of polymers. This phenomena, enabled flocculation by depletion of non-adsorbed polymers which may generate an osmotic attraction driving force between drops which has been reported to increase with polymer concentration so as to overcome repulsion forces between drops (Klinkesorn *et al*., 2004; Jafari

et al., 2007b; Yao *et al*., 2013) or by forming complexes that reduce surface activity (Wangsakan *et al*., 2004). PhC, on the other hand, may interact with GA possibly increasing its emulsifying capacity (Omar *et al*., 2009).

Figure 4b shows that pre-emulsions prepared by using surfactants had similar and greater FD's than emulsions without surfactant. In the emulsions containing SDS, FD changed with microfluidization cycles and FD values were statistically similar for emulsions with Tw and PhC and greater than those with SDS. Microfluidization increased FD for emulsions without surfactant, but the value of this parameter was similar to those of emulsions prepared by adding Tw and PhC. Values of Λ had an opposite trend than FD as shown in Figure 4c. Emulsion without surfactant and Tw had similar values of Λ when applying 1 and 2 processing cycles. Emulsions prepared by using SDS and PhC had the highest and lowest values of Λ, considering that these surfactants promotes a greater interaction among the micelles.

FD values were associated to contour irregularity of micelles or micellar agglomerates due to composition of emulsion and processing, *i.e*., such
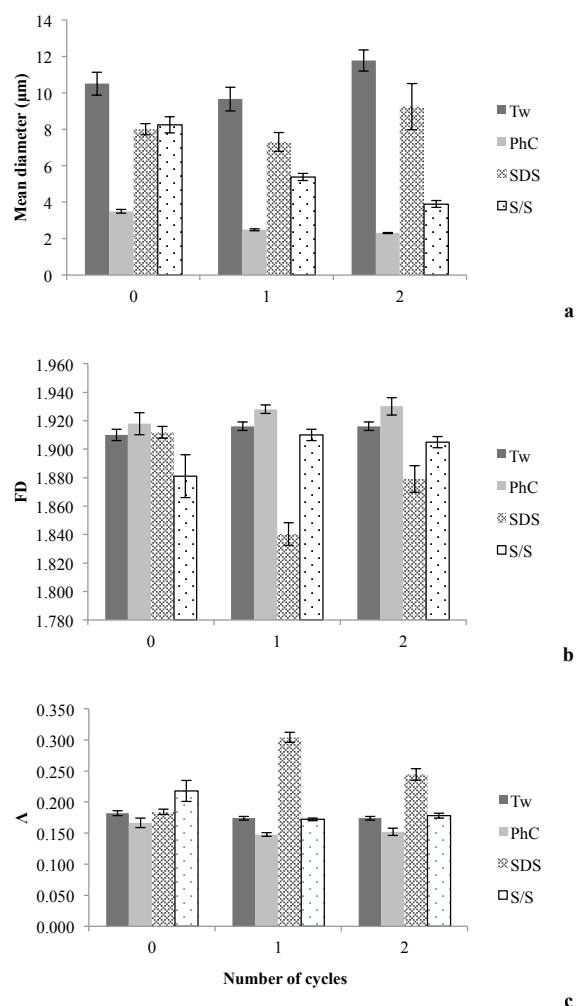
Fig. 4. Bar graphs corresponding to variation of mean diameter (a) FD (b) and Λ (c) for emulsions prepared with 0.80 % of surfactant. "0" is pre-emulsion.

irregularity would be defined by spatial arrangements of individual micelles of different sizes forming micellar agglomerates. On the other hand, Λ is related to agglomerate distribution and, in particular to their spatial dispersion. High Λ values were related to the formation of very complex structures that may provide evidence of destabilization of the emulsions by agglomeration. High Λ values have been associated to short shelf lives of yogurt (Lobato-Calleros *et al.*, 2009).

Figure 5, shows captured images for emulsions prepared by using 1.60 % of surfactant. Images show the formation of micellar agglomerates in emulsions prepared by using Tw and SDS. Emulsions prepared without surfactant and those containing PhC had a better dispersion at both concentrations of surfactant.

In Figure 6 (a,b and c), it is possible to observe size, FD and Λ of M/MA of the different emulsions prepared by adding 1.60% of the different surfactants. Maximum size of M/MA (7.51 μm) was observed for emulsions prepared with the addition of Tw while the minimum ones (3.31 μm) were obtained by using PhC as depicted in Figure 6a. In the emulsion without surfactant and in those with PhC and Tw, microfluidization decreased the mean diameter of emulsions. On the other hand, in emulsions with SDS, microfluidization increased the mean diameter of emulsions (Tcholakova *et al.*, 2004; Jafari *et al.*, 2008). Increasing the concentration of PhC (from 0.80 to 1.60%) did not cause significant changes ($p > 0.05$) in the sizes of the M/MA, however, when using Tw and SDS high surfactant concentrations reduced sizes of M/MA (Figure 4a and 6a).

Theoretical studies and experimental results indicate that when using turbulent shear and excess of surfactant ("rich in surfactant" regime), mean diameter of drops was mainly affected by energy inputs in the emulsification chamber and by the oil-water interfacial tension which indicated that mean size, largely depends on characteristics of surfactant (Taisne *et al.*, 1996). At the lower concentration of surfactants mean size, strongly depends on type and concentration of surfactant. It has been shown that mean size rapidly decreases with an increase of initial concentration of surfactant for this "poor in surfactant" regime. Other important effect of a surfactant is its interfacial adsorption rate which largely determines stabilization of newly formed drops against coalescence (Schulz and Daniels, 2000; Schultz *et al.*, 2004; Jafari *et al.*, 2008).

Figure 6b show that the pre-emulsions had similar FD values among each other and for emulsions containing Tw, FD value (1.88) did not change significantly ($p > 0.05$) with microfluidization cycles. Emulsions prepared with SDS and PhC and with no surfactant, had similar FD values among them. Microfluidization increase FD of emulsion with no surfactant and with PhC. In Figure 6c, it is possible to observe that Λ values of emulsions with no surfactant, with SDS and PhC was similar. Emulsion with Tw did no report statistic differences ($p > 0.05$) in Λ with microfluidization cycles.

The increase in surfactant concentration of surfactants did not generate structural changes in micelles prepared by the addition of PhC, since they presented similar FD, Λ and mean size values in contrast with emulsions prepared by adding Tw and SDS at both concentrations of these compounds.
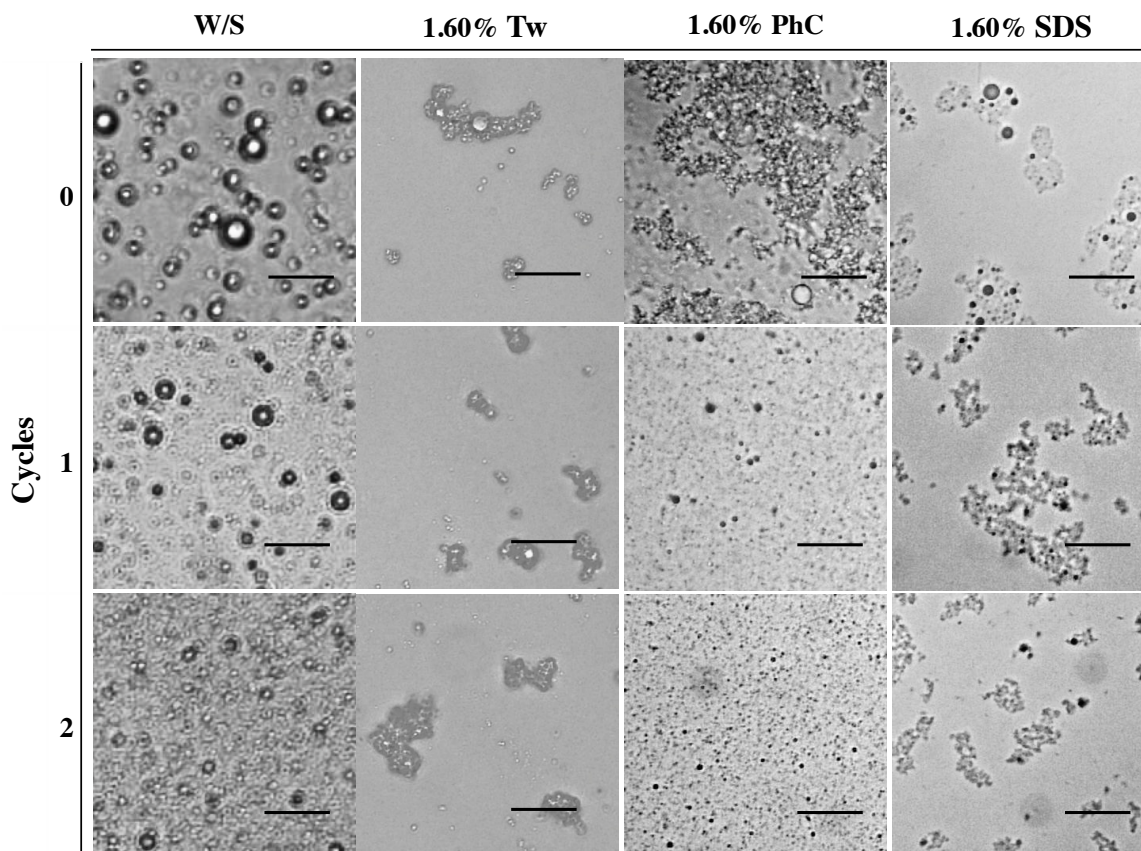
Fig. 5. Images (gray-scale) of prepared emulsions with different surfactants (concentration of 1.60%) and microfluidization cycles. "0" is pre-emulsion and "W/S" is without surfactant. Length of reference line is 50 μm.

3.2 Descriptive model of surfactant-morphology relationships (PCA analysis)

Figure 7, is a bubble graph presenting a descriptive model map of Λ and FD tendencies for the different formulations used. PCA indicated that significant contributions towards FD and Λ are due to Tw and SDS. Addition of different concentrations of PhC, on the other hand, did not generate significant differences ($p > 0.05$) in FD and Λ. High values of FD and low values of Λ were obtained when using PhC and the opposite for Tw and SDS regardless of concentration or the use of 1 or 2 cycles of microfluidization cycles.

Information given by FD and Λ complement to each other. Very small droplets contribute in a higher proportion to the reported values of FD than the larger ones since changes in the shape of contours of the small particles affect its irregularity at a greater extent than the changes in shape of large particles given the resolution of the image capturing system and the exactly equal procedure applied for processing each image. Hence, the effect of the presence of very small and very big droplets was, therefore, compensated.

FD, was related with micelle arrangements, conjunction of individual particles, the irregularity of each individual micelle and the amount of dispersed material forming the structure of the agglomerate whereas Λ was related with dispersion of particles which in turn may be related to electrokinetic potentials (Hunter, 1981). For example, in emulsions containing PhC, individual particles are the smallest and small irregular micellar agglomerates were, thus formed. In emulsions containing Tw, individual particles are greater in size, with larger and less compact agglomerates than those in emulsions with PhC and SDS, showing a high amount of individual particles, with a non-occupied fraction, which tend to form less homogeneous systems.
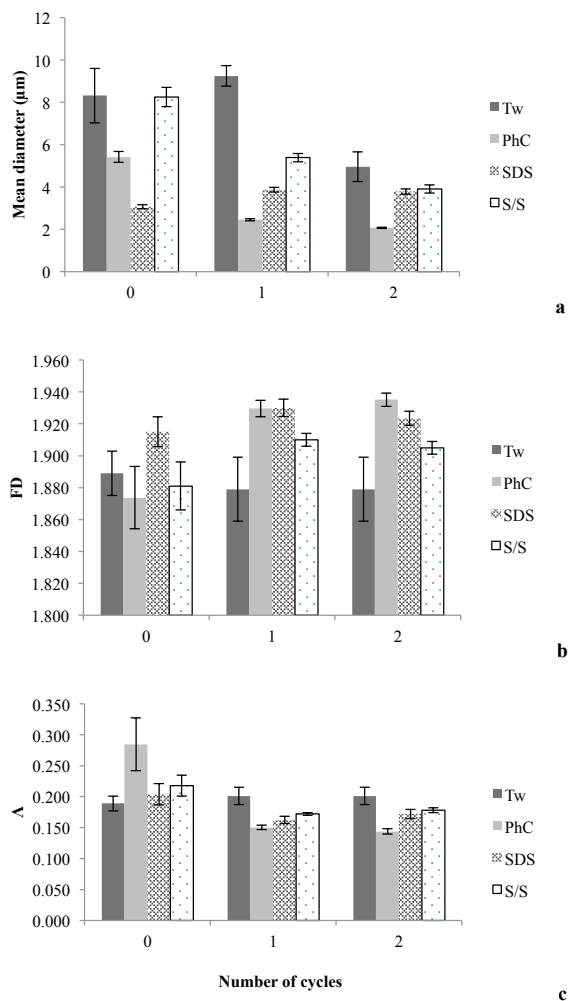
Fig. 6. Bar graphs corresponding to variation of mean diameter (a) FD (b) and Λ (c) for emulsions prepared with 1.60 % of surfactant. "0" is pre-emulsion.
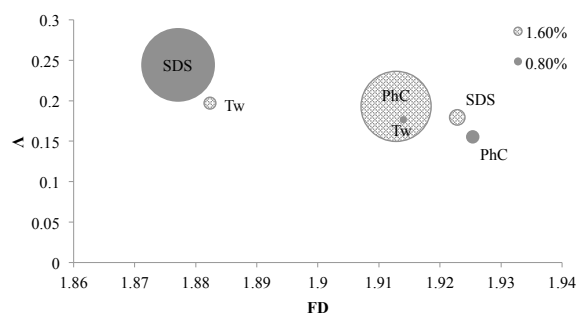

Fig. 7. Bubble graph showing Λ-FD relationships for the different surfactants used (Descriptive model of surfactant-morphology relationships).

In emulsions with SDS, individual particles are medium sized, having agglomerates with a lower quantity of compacted particles.

The different behavior observed for each surfactant in relation to particle size, contour irregularity and spatial dispersion homogeneity might be related to the ionic character of the surfactants as well as their HLB value. The aqueous phase would then hold MD and GA since these compounds had more affinity for water than for the AT. The surfactants used in this work have different HLB value (Griffin, 1949), being approximately 15, 12 and 7 for Tween 20, SDS and PhC, respectively so Tw 20 and SDS interact at a greater extent with the aqueous phase than with the oil phase (AT), which may explain the aggregation and the higher contour irregularity of AT agglomerates. The PhC, besides its cationic character, has higher affinity for the AT, which generates dispersed, smoother and small aggregates. PhC reduces permeation rate of free radicals through emulsion interface at a greater rate than other surfactants as Tw (Pan *et al.*, 2013).

Conclusions

The three surfactants induced the formation of micellar agglomerates, the contribution of these surfactants to macroscopic homogeneity of the emulsion was a function of concentration and microfluidization. Lecithin increased macroscopic homogeneity of emulsions regardless of the use of 1 or 2 cycles of microfluidization and its concentration. The lowest diameters of micelles were obtained with this surfactant, which resulted in a better dispersion and irregular micellar structures (high FD). Macroscopic homogeneity of emulsion was not influenced by smooth or irregular contours of micelles or micellar agglomerates. FD could be related to geometric homogeneity of micelles and was partially controlled by particle size and Λ was related with dispersion of micelles and their size. Image analysis technique provided valuable information on system behavior as related to composition and processing. Assessment of emulsion stability is a complex and multivariate task, which requires accumulated evidence from a number of experimental and theoretical analysis.

Acknowledgements

References

Abramoff, M.D., Magalhaes, P.J. and Ram, S.J. (2004). Image Processing with ImageJ. *Biophotonics International 11*, 36-42.

Barleita, B.J. and Barbosa-Canovas, G. (1993). Fractal analysis to characterize ruggedness changes in tapped agglomerated food powders. *Journal of Food Science 58*, 1030-1046.

Boom, R. M. (2008). Emulsions: principles and preparation. In: *Food Materials Science*, (J.M. Aguilera and P.J. Lillford, eds.), Pp. 305-339. Springer, New York.

Borel, P., Preveraud, D. and Desmarchelier, C. (2013). Bioavailability of vitamin E in humans: an update. *Nutrition Reviews 71*, 319-331.

Bramley, P., Elmadfa, I., Kafatos, A., Kelly, F., Manios, Y., Roxborough, H., Schuch, W., Sheehy, P. and Wagner, K.-H. (2000). Review Vitamin E. *Journal of the Science of Food and Agriculture 80*, 913-938.

Chaparro-Mercado, M.C., García-Ochoa, F., Hernández-Sánchez, H., Alamilla-Beltrán, L., Quintanilla-Carvajal, M.X., Cornejo-Mazón, M., Pedroza-Islas, R. and Gutierrez-López, G.F. (2012). Diseño de una estructura intersticial para una emulsión de aceite de semilla de uva por diseño de experimentos y superficie de respuesta. *Revista Mexicana de Ingeniería Química 11*, 11-21.

Chen, C.C. and Wagner, G. (2004). Vitamin E nanoparticle for beverage applications. *Chemical Engineering Research and Design 82*, 1432-1437.

Chiu, Y.C. and Yang, W.L. (1992). Preparation of vitamin-E microemulsion possessing high-resistance to oxidation in air. *Colloids and Surfaces 63*, 311-322.

Ciron, C.I.E., Gee, V.L., Kelly, A.L. and Auty, M.A.E. (2010). Comparison of the effects of high-pressure microfluidization and conventional homogenization of milk on particle size, water retention and texture of non-fat and low-fat yoghurts. *International Dairy Journal 20*, 314-320.

Dalgleish, D.G., Tosh, S.M. and West, S. (1996). Beyond homogenization: The formation of very small emulsion droplets during the processing of milk by a microfluidizer. *Netherlands Milk and Dairy Journal 50*, 135-148.

Dàvila, E. and Parés D. (2007). Structure of heat-induced plasma protein gels studied by fractal and lacunarity analysis. *Food Hydrocolloids 21*, 147-153.

Eitenmiller, R.R. and Lee, J. (2004). *Vitamin E: Food Chemistry, Composition, and Analysis*. Marcel Dekker, New York.

Feng, J.-L., Wang, Z.-W., Zhang, J., Wang, Z.-N. and Liu, F. (2009). Study on food-grade vitamin E microemulsions based on nonionic emulsifiers. *Colloids and Surfaces, A: Physicochemical and Engineering Aspects 339*, 1-6.

Friberg, S., Larsson, K. and Sjoblom, J. (2004). *Food Emulsions*. Marcel Dekker, New York.

Fujita, K., Iwasaki, M., Ochi, H., Fukuda, T., Ma, C., Miyamoto, T., Takitani, K., Negishi-Koga, T., Sunamura, S., Kodama, T., Takayanagi, H., Tamai, H., Kato, S., Arai, H., Shinomiya, K., Itoh, H., Okawa, A. and Takeda, S. (2012). Vitamin E decreases bone mass by stimulating osteoclast fusion. *Nature Medicine 18*, 589-594.

Gawrysiak-Witukska, M., Siger, A. and Nogala-Kalucka, M. (2009). Degradation of tocopherols during near-ambient rapeseed drying. *Journal of Food Lipids 16*, 524-539.

Gonnet, M., Lethuaut, L. and Boury, F. (2010). New trends in encapsulation of liposoluble vitamins. *Journal of Controlled Release 146*, 276-290.

Griffin, W.C. (1949). Classification of Surface Active Agents by HLB. *Journal of the Society of Cosmetic Chemists 1*, 311-326.

Hoppe, P.P. and Krennrich, G. (2000). Bioavailability and potency of natural-source and all-racemic α-tocopherol in the human: a dispute. *European Journal of Nutrition 39*, 183-93.

Hunter, R.J. (1981). *Zeta Potential in Colloid Science: Principles and Applications*. Academic Press, London.

Jafari, S.M., Assadpoor, E., He, Y. and Bhandari, B. (2008). Re-coalescence of emulsion droplets during high-energy emulsification. *Food Hydrocolloids 22*, 1191-1202.

Jafari, S.M., He, Y. and Bhandari, B. (2007a). Optimization of nano-emulsions production by microfluidization. *European Food Research and Technology 225*, 733-741.

Jafari, S.M., He, Y. and Bhandari, B. (2007b). Effectiveness of encapsulating biopolymers to produce sub-micron emulsions by energy emulsification techniques. *Food Research International 40*, 862-873.

Jafari, S.M., He, Y. and Bhandari, B. (2006). Nano-emulsion production by sonication and microfluidization: A comparison. *International Journal of Food Properties 9*, 475-485.

Karperien, A. (2004). *FracLac Advanced User's Manual*. Charles Sturt University, Australia.

Kenkel, N.C. and Walker, D.J. (1996). Fractals in the biological sciences. *Coenoses 11*, 77-100.

Klinkesorn, U., Sophanodora, P., Chinachoti, P. and McClements, D.J. (2004). Stability and rheology of corn oil-in-water emulsions containing maltodextrin. *Food Research International 37*, 851-859.

Kralova, I. and Sjoblom, J. (2009). Surfactants used in food industry: A review. *Journal of Dispersion Science and Technology 30*, 1363-1383

Lobato-Calleros, C., Recillas-Mota, M.T., Espinosa-Solares, T., Alvarez-Ramirez, J. and Vernon-Carter, E.J. (2009). Microstructural and rheological properties of low-fat stirred yoghurts made with skim milk and multiple emulsions. *Journal of Texture Studies 40*, 657-675.

Lopes, R. and Betrouni, N. (2009). Fractal and multifractal analysis: a review. *Medical Image Analysis 13*, 634-649.

Mandelbrot, B.B. (1983). *The Fractal Geometry of Nature*. WH Freeman and Co., New York.

Mayer, S., Weiss, J. and McClements, D.J. (2013a). Vitamin E-enriched nanoemulsions formed by emulsion phase inversion: Factors influencing droplet size and stability. *Journal of Colloid and Interface Science 402*, 122-30.

Mayer, S., Weiss, J. and McClements, D.J. (2013b). Behavior of vitamin E acetate delivery systems under simulated gastrointestinal conditions: Lipid digestion and bioaccessibility of low-energy nanoemulsions. *Journal of Colloid and Interface Science 404*, 215-22.

Meraz-Torres L. S., Quintanilla-Carvajal M.X., Téllez-Medina D.I., Hernández-Sánchez H., Alamilla-Beltrán L. and Gutiérrez-López G.F. (2011). Water droplet spreading and recoiling upon contact with thick compact maltodextrin agglomerates. *Journal of the Science of Food and Agriculture 91*, 2594-2600.

McClements, D.J. (2012). Nanoemulsions versus microemulsions: terminology, differences, and similarities. *Soft Matter 8*, 1719-1729.

McClements, D.J. (2011). Edible nanoemulsions: fabrication, properties, and functional performance. *Soft Matter 7*, 2297-2316.

McClements, D.J. (2005). *Food Emulsions: Principles, Practices, and Techniques*. CRC Press, Boca Raton.

McClements, D.J. (1994). Ultrasonic determination of depletion flocculation in oil-in-water emulsions containing a non-ionic surfactant. *Colloids and Surfaces A: Physicochemical and Engineering Aspects 90*, 25-35.

McClements, D.J., Decker, E.A., Park, Y. and Weiss, J. (2009). Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. *Critical Reviews in Food Science and Nutrition 49*, 577-606.

McClements, D.J. and Rao, J. (2011). Food-Grade nanoemulsions: Formulation, fabrication, performance, biological fate, and potential toxicity. *Critical Reviews in Food Science and Nutrition 51*, 285-330.

Microfluidics (2008). *Microfluidizer Processor User Guide*. Microfluidics Corp. USA: Newton, USA.

Montgomery, D.C. (1991). *Design and Analysis of Experiments*. Wiley and Sons, Inc., USA.

Omar, K.A., Shan, L., Zou, X., Song, Z. and Wang, X. (2009). Effects of Two emulsifiers on yield and storage of flaxseed oil powder by response surface methodology. *Pakistan Journal of Nutrition 8*, 1316-1324.

Pan, Y., Tikekar, R.V. and Nitin, N. (2013). Effect of antioxidant properties of lecithin emulsifier on oxidative stability of encapsulated bioactive compounds. *International Journal of Pharmaceutics 450*, 129-137.

Pantic, I., Paunovic, J., Basta-Jovanovic, G., Perovic, M., Pantic, S. and Milosevic, N.T. (2013). Age-related reduction of structural complexity in spleen hematopoietic tissue architecture in mice. *Experimental Gerontology 48*, 926-932.

Perrier-Cornet, J.M., Marie, P. and Gervais, P. (2005). Comparison of emulsification efficiency of protein-stabilized oil-in-water emulsions using jet, high pressure and colloid mill homogenization. *Journal of Food Engineering 66*, 211-217.

Petrovic, L., Sovilj, V., Katona, J. and Milanovic, J. (2010). Influence of polymer-surfactant interactions on o/w emulsion properties and microcapsule formation. *Journal of Colloid and Interface Science 342*, 333-339

Quintanilla-Carvajal, M.X., Meraz-Torres, L. S., Alamilla-Beltrán, L., Chanona-Pérez, J.J., Terres-Rojas, E., Hernández-Sánchez, H., Jiménez-Aparicio, A.R. and Gutiérrez-López, G. F. (2011). Morphometric characterization of spray-dried microcapsules before and after α-tocopherol extraction. *Revista Mexicana de Ingeniería Química 10*, 301-312.

Ricciarelli, R., Zingg, J. and Azzi, A. (2002). The 80 th Anniversary of Vitamin E: Beyond Its Antioxidant Properties. *Biological Chemistry 383*, 457-465.

Saberi, A.H., Fang, Y. and McClements, D.J. (2013). Fabrication of vitamin E-enriched nanoemulsions: factors affecting particle size using spontaneous emulsification. *Journal of Colloid and Interface Science 391*, 95-102.

Sagalowicz, L. and Leser, M.E. (2010). Delivery systems for liquid food products. *Current Opinion in Colloid and Interface Science 15*, 61-72.

Schneider, C. (2005). Chemistry and biology of vitamin E. *Molecular Nutrition and Food Research 49*, 7-30.

Schultz, S., Wagner, G., Urban, K. and Ulrich, J. (2004). High-pressure homogenization as a process for emulsion formation. *Chemical Engineering and Technology 27*, 361-368.

Schulz, M.B. and Daniels, R. (2000). Hydroxypropylmethylcellulose (HPMC) as emulsifier for submicron emulsions: Influence of molecular weight and substitution type on the droplet size after high-pressure homogenization. *European Journal of Pharmaceutics and Biopharmaceutics 49*, 231-236.

Schuster, S., Bernewitz, R., Guthausen, G., Zapp, J., Greiner, A.M., Köhler, K. and Schuchmann, H.P. (2012). Analysis of W1/O/W2 double emulsions with CLSM: Statistical image processing for droplet size distribution. *Chemical Engineering Science 81*, 84-90.

Taisne, L., Walstra, P. and Cabane, B. (1996). Transfer of oil between emulsion droplets. *Journal of Colloid and Interface Science 184*, 378-390.

Tansel, B. and Sevimoglu, O. (2006). Coalescence and size distribution characteristics of oil droplets attached on flocs after coagulation. *Water, Air, and Soil Pollution 169*, 293-302.

Tcholakova, S., Denkov, N.D. and Danner, T. (2004). Role of surfactant type and concentration for the mean drop size during emulsification in turbulent flow. *Langmuir 20*, 7444-7458.

Velazquez-Camilo, O., Bolaños-Reynoso, E., Rodriguez, E. and Alvarez-Ramirez, J. (2010). Characterization of cane sugar crystallization using image fractal analysis. *Journal of Food Engineering 100*, 77-84.

Veleva, L., García-González, A. and Pérez, G. (2013). Fractal quantification of aluminum pitting corrosion induced by humid tropical climate. *Revista Mexicana de Ingeniería Química 12*, 65-72.

Wangsakan, A., Chinachoti, P. and McClements D.J. (2004). Effect of surfactant type on surfactantmaltodextrin interactions: Isothermal titration calorimetry, surface tensiometry, and

ultrasonic velocimetry study. *Langmuir 20*, 3913-3919.

Wilde, P. (2000). Interfaces: their role in foam and emulsion behavior. *Current Opinion in Colloid and Interface Science 5*, 176-181

Yang, Y. and McClements, D.J. (2013a). Vitamin E and Vitamin E acetate solubilization in mixed micelles: Physicochemical basis of bioaccessibility. *Journal of Colloid and Interface Science 405*, 312-21.

Yang, Y. and McClements, D.J. (2013b). Encapsulation of vitamin E in edible emulsions fabricated using a natural surfactant. *Food Hydrocolloids 30*, 712-720.

Yang, Z. and Huffman, S.L. (2011). Review of fortified food and beverage products for pregnant and lactating women and their impact on nutritional status. *Maternal and Child Nutrition 7*, 19-43.

Yao, X., Wang, N., Fang, Y., Phillips, G.O., Jiang, F., Hu, J., Lu, J., Xu, Q. and Tian, D. (2013). Impact of surfactants on the lipase digestibility of gum arabic-stabilized O/W emulsions. *Food Hydrocolloids 33*, 393-401.

Zingg, J.M. and Azzi, A. (2004). Non-antioxidant activities of vitamin E. *Current Medicinal Chemistry 11*, 1113-1133.

Zúñiga, R.N., Skurtys, O., Osorio, F., Aguilera, J.M. and Pedreschi, F. (2012). Physical properties of emulsion-based hydroxypropyl methylcellulose films: Effect of their microstructure. *Carbohydrate Polymers 90*, 1147-1158.