CARACTERÍSTICAS MICROMORFOMÉTRICAS DE EMULSIONES DE α-TOCOPHEROL OBTENIDAS POR MICROFLUIDIZACIÓN

MICROMORPHOMETRIC CHARACTERISTICS OF α-TOCOPHEROL EMULSIONS OBTAINED BY MICROFLUIDIZATION

C. Cano-Sarmiento¹, A. Monroy-Villagran¹, L. Alamilla-Beltrán¹, H. Hernández-Sánchez¹, M. Cornejo-Mazón², D.I. Téllez-Medina¹, C. Jiménez-Martínez¹ and G.F. Gutiérrez-López¹*

²Departamento de Biofísica, Escuela Nacional de Ciencias Biológicas-IPN.

Received November 22, 2013; Accepted December 5, 2013

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 independientemente 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 Krenrich, 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-Witkuska 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 frangere 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 (A) 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 (Dávila 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 A 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.

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, φ =75 µm) and the secondary one of the type “Z” (H30Z, φ = 200 µm).
Table 1. Formulation of preemulsion and emulsions at 1 and 2 microfluidization cycles

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (%(w/w))</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-T</td>
<td>2.83</td>
</tr>
<tr>
<td>MD</td>
<td>4</td>
</tr>
<tr>
<td>GA</td>
<td>4</td>
</tr>
<tr>
<td>Tw, PhC, SDS</td>
<td>0, 0.80 and 1.60</td>
</tr>
<tr>
<td>Water type I</td>
<td>89.17, 88.37 and 87.57</td>
</tr>
</tbody>
</table>

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 Sevimoglou, 2006; Zúñiga et al., 2012; Schuster et al., 2012). 5 fields of view were observed in an optical microscope Nikon H550S (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; Dávila 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.
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
irregularity would be defined by spatial arrangements of individual micelles of different sizes forming micellar agglomerates. On the other hand, \( \Lambda \) is related to agglomerate distribution and, in particular to their spatial dispersion. High \( \Lambda \) values were related to the formation of very complex structures that may provide evidence of destabilization of the emulsions by agglomeration. High \( \Lambda \) 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 \( \Lambda \) of M/MA of the different emulsions prepared by adding 1.60% of the different surfactants. Maximum size of M/MA (7.51 \( \mu \)m) was observed for emulsions prepared with the addition of Tw while the minimum ones (3.31 \( \mu \)m) 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 \( \Lambda \) values of emulsions with no surfactant, with SDS and PhC was similar. Emulsion with Tw did no report statistic differences (\( p > 0.05 \)) in \( \Lambda \) 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, \( \Lambda \) and mean size values in contrast with emulsions prepared by adding Tw and SDS at both concentrations of these compounds.
Figure 5. Images (gray-scale) of prepared emulsions with distinct 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.
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**

Author CCS, is grateful to CONACyT and IPN-Mexico for study grant. Authors thank financial
support by CONACyT and IPN-Mexico for carrying out this work.

References


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.


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


