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Rheological properties of a double emulsion nutraceutical system incorporating chia essential oil and ascorbic acid stabilized by carbohydrate polymer–protein blends

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ABSTRACT

Four water-in-oil-in water ($W_1/O/W_2$) double emulsions were made by adding the primary emulsion (W_1/O) containing 74% (w/w) of chia essential oil, 6% (w/w) of ascorbic acid, and a 0.2 dispersed phase mass fraction ($\varphi_{W_1/O}$) to aqueous solutions (W_2) of mesquite gum (MG), maltodextrin DE-10 (MD) and whey protein concentrate (WPC) in different proportions (MG66–MD17–WPC17 and MG17–MD66–WPC17), and in a ratio of 1:2.12 and 1:4.12 W_1/O to dry biopolymers blends solids. All the double emulsions showed type C morphologies and only slight changes in the volume-weighted mean diameter ($d_{4,3}$) throughout the storage time, indicative of good stability, despite they presented bimodal size distributions, but the double emulsion formulated with a predominant proportion of MD and ratio 1:2.12 provided a higher stability against droplet coalescence. All the double emulsions displayed viscoelastic character dependent on frequency.

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1. Introduction

In recent years the trend in the market for functional foods or nutraceuticals has increased, and further increase in the development and consumption of these products is forecasted, particularly for products that include in their formulation functional and natural food additives like essential oils, vitamins, minerals, carotenoids, flavonoids, anthocyanins, etc. (Torres-Giner, Martinez-Abad, Ocio, & Lagaron, 2010), with high nutritional value, and that may contribute to prevent cardiovascular, respiratory and carcinogenic diseases, among others (Shahidi, 2009).

Chia (*Salvia hispanica* L.) essential oil has significantly higher content of α-linolenic and linoleic acids (Álvarez-Chávez, Valdivia-López, Aburto-Juárez, & Tecante, 2008; Ayerza, 1995), than linseed, canola and soybean oils (Gunstone & Padley, 1997). It is known that α-linolenic acid (Omega 3) is essential in the diet, and because the human body cannot synthesize it, it is necessary to include it through diet. Several studies have shown that Omega 3 (ω-3) provides multiple health benefits including cardiovascular risk reduction, prevention of diseases of the nervous system and decreased symptoms of inflammatory diseases such as rheumatoid arthritis (Djordjevic, McClements, & Decker, 2004; Shahidi, 2006).

On the other hand, ascorbic acid (AA) is one of the most important vitamins, often added in the trade of fortified formulas (Sablani, Al-Belushi, Al-Marhubi, & Al-Belushi, 2007), acting as a water soluble antioxidant, with the capacity of stabilizing free radicals, preventing cardiovascular and degenerative diseases, among others (Pénicaud, Peyron, Bohuon, Gontard, & Guillard, 2010).

Water-in-oil-in-water ($W_1/O/W_2$) double emulsions offer several strategic advantages for use in food applications: (i) they allow to incorporate separately oil- and water-soluble sensitive ingredients in a single system (Muschiolik, 2007; Rodríguez-Huezo, Pedroza-Islas, Prado-Barragán, Beristain, & Vernon-Carter, 2004); (ii) the encapsulated ingredients can be delivered at a controlled rate during eating and digestion (Dickinson, 2011); (iii) the sensitive compounds can be isolated from entering in contact with detrimental environmental factors that may degrade them through the design of protective membranes (McClements, Decker, & Weiss, 2007). Still, one of the main challenges for the food scientist is

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to produce stable $W_1/O/W_2$ double emulsions with food-grade emulsifiers and stabilizers instead of the synthetic surfactants and polymers used in other industries. Multiple emulsions offer advantages for application in the food industry, as double emulsions are much more difficult to prepare and characterize than simple emulsions, and their stability is more difficult to maintain (Dickinson, 2011). $W_1/O/W_2$ double emulsions tend to exhibit the best stability when containing substantial number flocculated, rather close-packed, inner droplets (Dickinson & McClements, 1996). Water-in oil primary emulsions prepared under high shear conditions with these characteristics are usually made with a blend of oil- and water-soluble emulsifiers, with the former in excess (Lobato-Calleros, Rodríguez, Sandoval-Castilla, Vernon-Carter, & Alvarez-Ramírez, 2006; Lobato-Calleros et al., 2008). Long term stability of the primary emulsion may be improved by incorporating a thickening or gelling polymer within the inner aqueous phase (Rodríguez-Huezo et al., 2004). Stable $W_1/O/W_2$ double emulsions have been obtained by using several proteins and/or carbohydrate polymers in the outer aqueous phase, some or all of them with surface active properties (Jiménez-Alvarado, Beristain, Medina-Torres, Román-Guerrero, & Vernon-Carter, 2009; Lobato-Calleros et al., 2006, 2008). The adsorbed layers formed around the $W_1/O/W_2$ double emulsions will depend on the nature of the biopolymers and on how these biopolymers are incorporated unto the interface (Bergenståhl, 1995), i.e., by associative adsorption or layered adsorption, or as individual molecules or as biopolymer complexes. Thickness of the adsorbed biopolymer layer affects overall functionality of $W_1/O/W_2$ double emulsions, and the greater the thickness, the higher the emulsion stability, better the encapsulation efficiency, increased protection against detrimental environmental factors, and slower release rates of encapsulated bioactive compounds (Jiménez-Alvarado et al., 2009).

The aim of this work was to: (a) produce $W_1/O/W_2$ double emulsions, incorporating in the inner aqueous phase ascorbic acid and in the intermediate oil phase chia essential oil, stabilized the associative adsorption of mesquite gum and maltodextrin DE-10 (carbohydrate polymers) and whey protein concentrate ternary blends on the outer oil–water interface; and (b) to determine the flow and viscoelastic properties, and the volume-weighted mean diameter of the double emulsions.

2. Materials and methods

Chia seeds (*Salvia hispanica* L.) were provided by farmers in the region Atlixco, State of Puebla, Mexico. A Tamer hydraulic press (Model PT-20, Shanghai, China) fitted with a 40 cm long by 10 cm of diameter plunger was used for cold pressing the chia seeds for obtaining the chia essential oil (CEO). Maximum pressure applied by the piston was 8.8×10^8 N m^{-2} to the piston, at room temperature. Trace amounts of seed were removed from CEO using a cloth filter, and filtered CEO was stored in amber bottles at a temperature of ~4 °C until required. Ascorbic acid (AA) with 99.8% purity was purchased from Sigma–Aldrich Quimica S.A. de C.V. (Toluca, State of Mexico, Mexico). Panodan SDK (esters of monoglycerides and diglycerides of diacetyl tartaric acid), a water-soluble surfactant (WS), and Grindsted PGPR 90 (esters of polyglycerol and polyricinoleate fatty acids) an oil-soluble surfactant (OS), were both purchased from Dannova Quimica S.A. de C.V. (Mexico, D. F., Mexico). Gellan gum (GG) provided by Merck & Co. (Kelco Division, Rahway, NJ, USA); mesquite gum (MG), hand collected in the form of tear drops from *Prosopis laevigata* trees in the Mexican State of San Luis Potosi and purified as indicated by Vernon-Carter et al. (1996); Maltodextrin DE-10 (MD) (Maltadex 10) was obtained from Complementos Alimenticios, S.A. de C.V. (Naucalpan, State of Mexico, Mexico); and whey protein concentrate (WPC; Hilmar 8000) containing 80% protein in dry basis was acquired from Hilmar Ingredients (Hilmar, CA, USA), were the selected biopolymers employed in this work. Deionized water was used in all the experiments, and sodium azide (Hycel de Mexico, S.A. de C.V., Mexico, D.F., Mexico) was used as preservative.

2.1. Formulation and preparation of the $W_1/O/W_2$ double emulsions

$W_1/O/W_2$ double emulsions were prepared at room temperature (25 ± 1 °C) using a two-stage emulsification procedure (Rodríguez-Huezo et al., 2004). In the first stage, a W_1/O emulsion was formulated with a 0.2 dispersed phase mass fraction ($\varphi_{W_1/O}$). Total emulsifier concentration in W_1/O was 8% (w/w) (one part of WS to four parts of OS). The inner aqueous phase W_1 contained 30% (w/w) of AA, 0.50% (w/w) of GG, and 8% (w/w) of WS. The inner aqueous phase (distilled water + WS + GG + AA) was added drop-wise to the oil phase (O) (CEO + OS) using an Ultra-Turrax T50 Basic homogenizer (IKA®-WERKE Works Inc., Wilmington, NC, USA) at 5200 rpm for 5 min. In the second stage the W_1/O primary emulsion was re-emulsified in aqueous solutions of ternary biopolymers blends in different proportions (MG66-MD17–WPC17 and MG17–MD66–WPC17). Based on the results obtained by Pérez-Alonso et al. (2008), these two protective colloid blends displayed relatively high activation energies and could provide high stability to the nutraceutical system. These biopolymers proportion were selected because it has been reported that when the outer droplets are made too small, in the process of secondary emulsification there is also a disruption of the internal aqueous droplets (Dickinson & McClements, 1996) For these reasons, the selection of the predominant biopolymers proportion on the blend was done based on their surface activity. Both, MG and MD possess significantly lower surface activity than WPC. In one case, the ratio of W_1/O to biopolymers solids was 1:2.12 and in the second case it was 1:4.12, yielding $W_1/O/W_2$ double emulsions with $\varphi_{W_1/O/W_2}$ of 0.13 and 0.075, respectively. Dispersion of the W_1/O emulsions in the aqueous solutions of the biopolymers blends was done using an Ultra-Turrax T50 Basic homogenizer (IKA®-WERKE Works Inc., Wilmington, NC, USA) at 7600 rpm for 6 min. The resulting emulsions were coded E_y^x, where x = the ratio of W_1/O to biopolymers solids in the outer aqueous phase (W_2), and y = biopolymers proportions in blend.

2.2. Characterization of the E_y^x emulsions

2.2.1. W_1/O and E_y^x emulsions droplet size

The volume-weighted mean diameter ($d_{4,3} = \sum n_i d_i^4 / \sum n_i d_i^3$), where n_i is the number of droplets with diameter d_i, of the W_1/O and of E_y^x emulsions upon formation and after refrigerated storage (4 °C) in amber flasks for 5 months was measured using a dynamic light scattering Malvern Particle Size Analyser (Mastersizer, 2000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). Refractive indexes of 1.475 and 1.333 were used for the W_1/O and E_y^x emulsions, respectively.

2.3. Rheological properties for the E_y^x emulsions

A Kinexus Pro rheometer (Malvern Instruments, Ltd, Worcestershire, UK), with a cone-plate geometry, in which the rotating cone was 40 mm in diameter, and cone angle of 4° was used for performing all the rheological measurements. Samples of E_y^x emulsions were carefully placed in the measuring system, and left to rest for 15 min for structure recovery and temperature equilibration. All the measurements were performed at 25 °C. Rheological characterization was carried out using steady shear and dynamic shear tests.

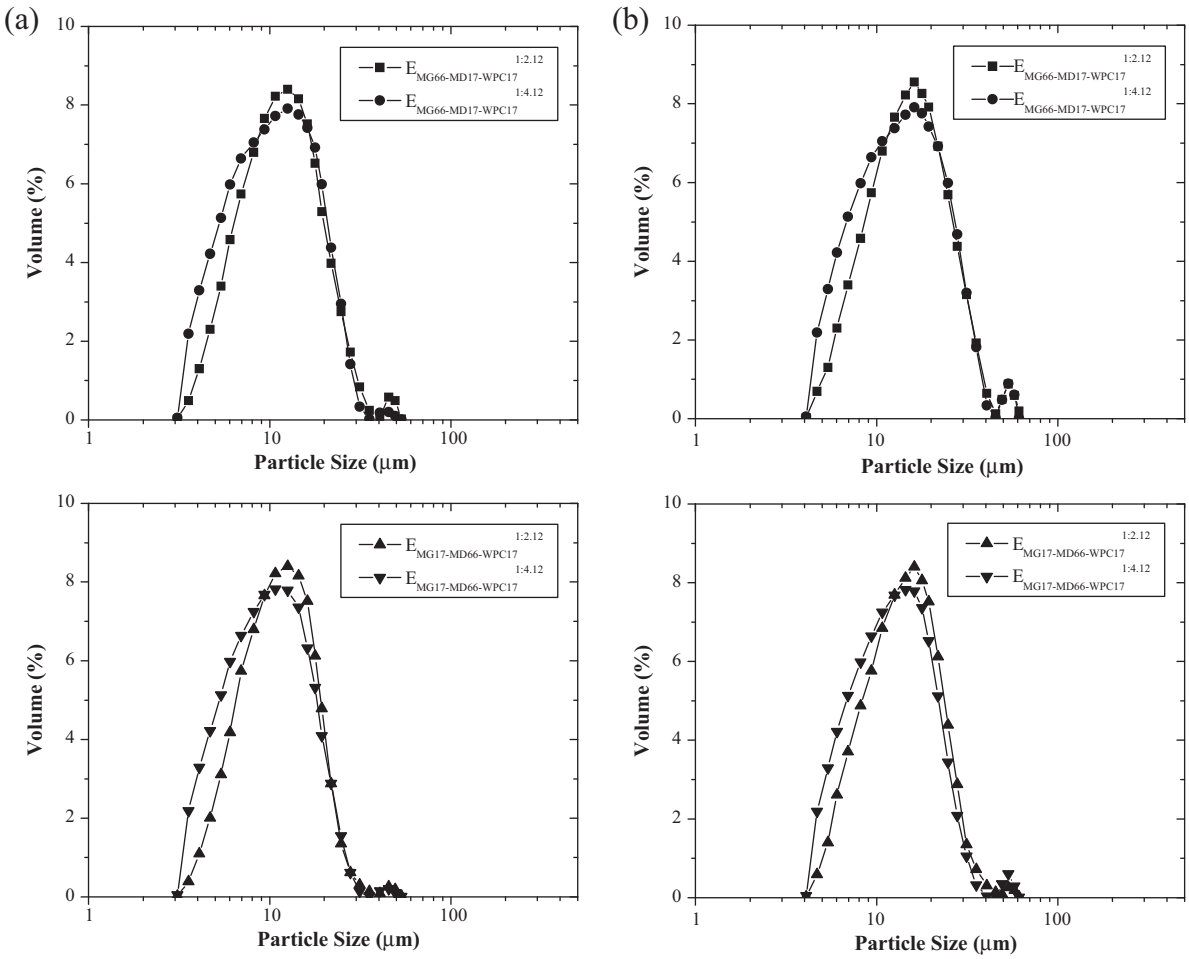

Fig. 1. Particle size distribution of the E_y^x emulsions: (a) fresh; (b) after 5 months storage.

2.3.1. Viscosity of E_y^x emulsions

The viscosity of each E_y^x emulsion was determined by applying an increasing shear rate from 0.1 to 100 s^{-1} and the apparent viscosity was recorded as a function of shear rate. Determination of the viscosity curve over a wide range of shear rate values is essential for solving the flow equations. This is usually done by fitting the data points to one of the viscosity models.

2.3.2. Dynamic shear rheological properties of the E_y^x emulsions

Dynamic amplitude sweeps (0.1–100% strain) were performed under a constant frequency (1 Hz), in order to determine the linear viscoelastic region (LVR), where rheological properties are not strain or stress dependent. From the LVR a strain level of 1% was chosen to perform frequencies sweeps at 0.1–100 rad s^{-1}. The storage modulus (G'), and the loss modulus (G'') were recorded as a function of frequency (Espinosa-Andrews, Sandoval-Castilla, Vázquez-Torres, Vernon-Carter, & Lobato-Calleros, 2010; Murillo-Martínez, Pedroza-Islas, Lobato-Calleros, Martínez-Ferez, & Vernon-Carter, 2011). In all cases, the data was analyzed using the equipment's rSpace version 2.00 software. These parameters represent the elastic and viscous components of the E_y^x emulsions, respectively. All measurements were done in triplicate.

2.4. Statistical analyses

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

3. Results and discussion

3.1. Characterization of the E_y^x emulsions

The freshly made primary W_1/O emulsion exhibited an monodispersed droplet size distribution (data not shown) with a $d_{4,3}$ of 0.29 ± 0.01 μm, and both, the droplet size distribution and $d_{4,3}$ remained practically unchanged up to 5 months storage time. On the other hand, all the freshly made E_y^x emulsions exhibited bimodal size distributions (Fig. 1a), and the droplet size distributions of all the emulsions tended to shift towards higher droplet sizes but remained bimodal (Fig. 1b). The ratio of W_1/O to biopolymers solids in the outer aqueous phase (W_2) had an effect on $d_{4,3}$ of the E_y^x emulsions. The emulsion made with a 1:4.12 ratio exhibited smaller droplet sizes than those made with a 1:2.12 ratio (Table 1). Surprisingly, the emulsions made with a predominant proportion of MD, not known for possessing a significant surface activity (Kenyon, 1995), displayed smaller $d_{4,3}$ than those made with a predominant proportion of MG, considered an effective emulsifying agent when used in relatively high concentrations (Vernon-Carter et al., 1996), probably because of the different adsorption structures that were formed at the outer oil–water interface. All the emulsions showed an increase in $d_{4,3}$ that ranged from 24.98 to 33.47 percent during the 5 month storage time (Table 1). Furthermore, all the emulsions were character-

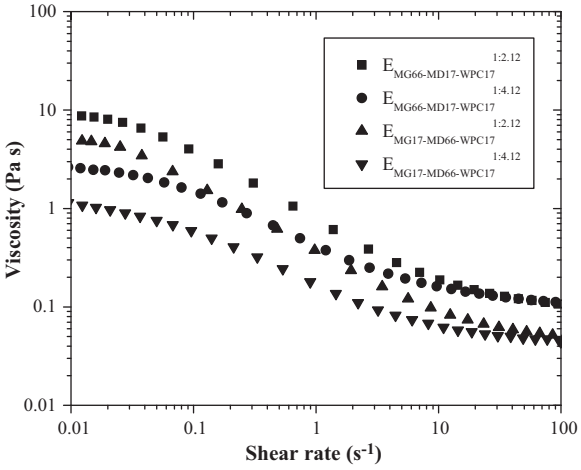

Fig. 2. Changes in the apparent viscosity as a function of shear rate for the E_y^x emulsions.

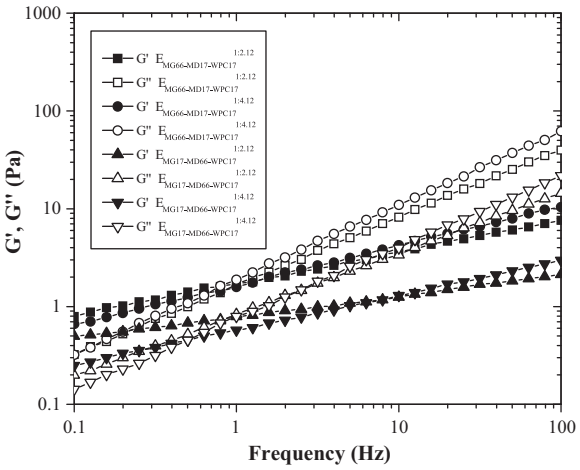

Fig. 3. Changes in storage (G') and loss (G'') modulus as a function of frequency sweep at 1% strain of the E_y^x emulsions.

ized by having C type morphologies, i.e., had substantial number of inner doplets which were maintained throughout the assay (Pimentel-González, Campos-Montiel, Lobato-Calleros, Pedroza-Islas, & Vernon-Carter, 2009). These results allow us to imply that the biopolymers indeed tended to associatively adsorb at the interface forming strong interfacial films that provided the E_y^x emulsions with relatively long term stability against coalescence, mainly due to repulsive force between the droplets by steric stability mechanisms (Vernon-Carter, Pedroza-Islas, & Beristain, 1998).

3.2. Rheological properties for the E_y^x emulsions

3.2.1. Viscosity of E_y^x emulsions

Fig. 2 shows that all the E_y^x emulsions presented a shear-thinning behavior as a result of the strain applied. When the magnitude of the shear rate was increased, the droplets of emulsions began to elongate to a greater extent in the direction of flow, resulting in a decrease in the viscosity of the system (shear-thinning or pseudoplastic character). This typical viscosity characteristic of many non-Newtonian fluids (e.g. polymeric fluids, flocculated dispersions, colloids) can be attributed to a reversible "structure" or network that forms in the "rest" or equilibrium state. When the material is sheared, the structure breaks down, resulting in a shear-dependent behavior (Logaraj, Bhattacharya, Sankar, & Venkateswaran, 2008). The experimental data of log viscosity versus log shear rate were analyzed with several equations (Casson, Cross, Carreau–Yasuda, modified Carreau, Carreau) that represent this "structural viscosity" type of behavior. The model that best fitted the experimental data in all cases was the Carreau rheological model ($R^2 \geq 0.995$) (Rao, 1999):

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{[1 + (\lambda\dot{\gamma})^2]^n} \quad (1)$$

where η is the apparent viscosity (Pa s), $\dot{\gamma}$ is the shear rate (s^{-1}), η_0 (Pa s) is the low shear rate limiting viscosity, η_∞ (Pa s) is the high shear rate limiting viscosity, λ (s) is a time constant related to the relaxation times of the flocculated E_y^x emulsions, and n (dimensionless) is the power-law behavior index. The values of these four parameters are given in Table 2 and their trends are very difficult to explain, as $d_{4,3}$, droplet size distribution, and the nature of the adsorbed biopolymer layers around the E_y^x emulsions seem to affect their outcome. In general terms, the E_y^x displayed higher η_0 and n values than their E_y^x counterparts.

Table 1
Volume-weighted mean diameter ($d_{4,3}$) of the E_y^x emulsions during storage.

Emulsion	t (months)	$d_{4,3}$ (μm)	Span (dimensionless)	Increase $d_{4,3}$ (%)
$E^{1:2.12}_{MG66-MD17-WPC17}$	0	13.49 ± 0.61^c	9.88 ± 0.44^b	30.69
	5	17.63 ± 0.79^e	13.12 ± 0.59^c	$\pm 1.38^c$
$E^{1:4.12}_{MG66-MD17-WPC17}$	0	12.31 ± 0.61^b	10.15 ± 0.51^b	33.47
	5	16.43 ± 0.82^e	14.02 ± 0.70^c	$\pm 1.67^c$
$E^{1:2.12}_{MG17-MD66-WPC17}$	0	12.65 ± 0.57^c	7.67 ± 0.34^a	24.98
	5	15.81 ± 0.71^e	12.51 ± 0.56^c	$\pm 1.12^a$
$E^{1:4.12}_{MG17-MD66-WPC17}$	0	11.41 ± 0.57^a	9.07 ± 0.45^b	27.34
	5	14.53 ± 0.73^d	13.96 ± 0.70^c	$\pm 1.37^b$

Superscripts with different letters in same column indicate significant differences ($P \leq 0.05$).

Table 2
Carreau model rheological parameters of the E_y^x emulsions.

Emulsion	η_0 (Pa s)	η_∞ (Pa s) × 10^2	λ (s)	n (dimensionless)	R^2
$E^{1:2.12}_{MG66-MD17-WPC17}$	9.21 ± 0.41^d	6.00 ± 0.30^c	34.11 ± 1.53^b	0.35 ± 0.02^b	0.999
$E^{1:4.12}_{MG66-MD17-WPC17}$	2.61 ± 0.13^b	7.00 ± 0.30^d	27.04 ± 1.35^a	0.29 ± 0.01^a	0.999
$E^{1:2.12}_{MG17-MD66-WPC17}$	5.23 ± 0.23^c	5.00 ± 0.20^b	35.84 ± 1.61^b	0.40 ± 0.02^c	0.999
$E^{1:4.12}_{MG17-MD66-WPC17}$	1.14 ± 0.06^a	2.00 ± 0.10^a	41.98 ± 2.10^c	0.25 ± 0.01^a	0.998

Superscripts with different letters in same column indicate significant differences ($P \leq 0.05$).

3.2.2. Dynamic shear rheological properties of the E_y^x emulsions

The changes in G' and G'' as a function of frequency of the E_y^x emulsions are shown in Fig. 3. Both, G' and G'' increased as the frequency increased, indicating that the emulsions displayed weak gel-like structure. Irrespectively of the W_1/O to biopolymers solids ratio in the E_y^x emulsions, G' showed higher values than G'' at low frequencies (<1 Hz), indicating a predominant solid-like behavior, but at high frequencies (>1 Hz) a cross-over between these parameters occurred, with behavior shifting to a predominant liquid-like one, indicating breakdown of the E_y^x emulsions structure (Fig. 3) (Hemar, Hall, & Singh, 2005).

4. Conclusions

All the $W_1/O/W_2$ double emulsions showed type C morphologies were maintained throughout the storage time and exhibited a high stability because of the relatively low increase in the volume-weighted mean diameter ($d_{4,3}$). The emulsions presented shear thinning behavior and viscoelastic character dependent of the frequency. The $E^{1:2.12}_{MG17-MD66-WPC17}$ emulsion provided a higher stability against increases in $d_{4,3}$. This study provides the bases for designing nutraceutical systems based on stable double emulsions.

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