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Preparation and characterization of water-in-oil emulsions loaded with high concentration of L-ascorbic acid

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ABSTRACT

The present study was conducted to encapsulate higher concentration of L-ascorbic acid up to (30 g 100 mL^{-1}) in the dispersed phase of water-in-oil (W/O) emulsions. Their continuous phase contained refined soybean oil or *Moringa oleifera* oil and a food-grade hydrophobic emulsifier. The volume fraction of the dispersed phase was fixed as to 30%. W/O emulsions with L-ascorbic acid retention greater than 95% were prepared using rotor–stator homogenizer at 7000 rpm for 5 min. The prepared W/O emulsions under this operating conditions had average droplet diameter of 2.0–3.0 μm and coefficients of variation of 13%–22%. All the W/O emulsions were stable for more than 30 days at 4 °C or 25 °C with slight increase in average droplet diameter and without phase separation. Their L-ascorbic acid retentions were 50 g 100 g^{-1} at 4 °C and 30 g 100 g^{-1} at 25 °C after 30 days of storage. L-ascorbic acid retention ratio of the prepared W/O emulsions followed first-order kinetics with a good fit.

1. Introduction

Emulsions are defined as dispersions of droplets of one liquid in another, with a droplet diameter generally within the range of 0.1–100 μm (Leal-Calderon, Schmitt, & Bibette, 2007). According to the definition of International Union of Pure and Applied Chemistry (IUPAC), "In an emulsion liquid droplets and/or liquid crystals are dispersed in a liquid". An emulsion consists of at least two immiscible phases such as oil and water, with one of the phases dispersed in the other (McClements, 2004). A dispersion consisting of oil droplets dispersed in an aqueous phase is called an oil-in-water (O/W) emulsion. O/W food emulsions include mayonnaise, milk, soups and sauces. On the other hand, a dispersion consisting of water droplets dispersed in an oil phase is called as water-in-oil (W/O) emulsion. Typical examples of W/O food emulsions are margarine, butter, and spreads.

L-ascorbic acid is a white, crystal-shaped organic compound, and can be synthesized from glucose or extracted from certain natural sources such as orange juice. Vitamin C plays a vital role in our body synthesis of collagen tissue around bones, teeth, cartilage, skin, and damaged tissue (Gallarate, Carlotti, Trotta, & Bovo, 1999). Similarly, this vitamin is needed to activate various enzymes related to the nervous system, hormones, and detoxification of drugs and poison in the liver (Lee et al., 2004). L-ascorbic acid plays a role as antioxidant, as its solubility in water enables it to work as antioxidant within our body fluids. Vitamin C increases the rate of absorption of iron, calcium, and folic acid. Vitamin C reduces allergic reactions, boosts the immune system, stimulates the formation of bile in the gallbladder, and facilitates the excretion of various steroids (Abbasi & Niakousari, 2007). Because of these favorable effects, vitamin C has been used in cosmetics and pharmaceutical applications (Pinnell et al., 2001). However its rapid degradation in aqueous solutions is still a major drawback to design a variety of functional products.

Vitamin C is highly soluble in water and alcohol, and is easily oxidized in the solubilized form and its oxidation occurs very quickly in an alkaline environment especially at higher temperatures (>50 °C) (Yuan & Chen, 1998). Similarly, light and heat damage vitamin C in fruits and vegetables (Ahmad et al., 2011). Degradation of vitamin C proceeds both by aerobic and anaerobic pathways and depends upon many factors such as oxygen, heat, light, storage temperature, and storage time (Nunes, Brecht, Morais, & Sargent, 1998; Satoh, Sakagami, Terasaka, Ida, & Fujisawa, 2000). The degradation processes of L-ascorbic acid are very complex and contain a number of oxidation/reduction with some intermolecular rearrangements (Kimoto, Tanaka, Ohmoto, & Choami, 1993; Lee et al., 2004). Fig. 1 shows pathway of L-ascorbic acid degradation in aqueous solution (Yuan & Chen, 1998). Degradation of L-ascorbic

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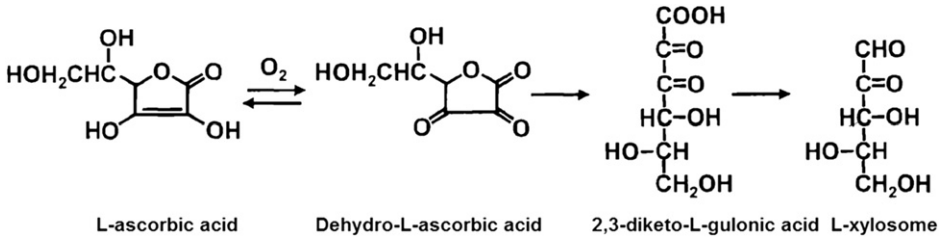

Fig. 1. Degradation pathways of L-ascorbic acid in aqueous solution (adopted from Lee et al., 2004).

acid results in brownish color possibly due to interaction with amino acids (Lee et al., 2004).

L-ascorbic acid is very unstable in aqueous solution. Encapsulation and emulsification are widely used techniques for protection of this vital vitamin (Farahmand, Tajerzadeh, & Farboud, 2006; Uddin, Hawlader, & Zhu, 2001). In most cases 1–5% of this vitamin is emulsified and encapsulated (Farahmand et al., 2006; Lee et al., 2004). Both W/O and water-in-oil-in-water (W/O/W) emulsification methods have been used for encapsulating vitamin C (Akhtar et al., 2010; Farahmand et al., 2006; Gallarate et al., 1999; Lee et al., 2004; Xia & Yao, 2011). In these emulsions the interface acts as a barrier for oxygen to prevent encapsulated vitamin C from undergoing oxidation induced degradation.

Foreseeing higher biological activity of L-ascorbic acid, the authors formulated W/O emulsions containing high concentration of L-ascorbic acid. The main purpose of this work is to elucidate that, up to what extent stable W/O emulsions can be prepared by incorporating higher amount of L-ascorbic acid. The effect of homogenization speed, concentration of L-ascorbic acid solubilized in a dispersed phase, stability of prepared emulsions, and retention kinetics of L-ascorbic acid in the prepared emulsions were also investigated.

2. Materials and methods

2.1. Materials

L-ascorbic acid (99.9% purity), magnesium sulfate, sodium bicarbonate, acetic acid, 2,6-dichlorophenol indophenol, metaphosphoric acid were purchased from Wako Pure Chemical Ind. (Osaka, Japan). All of these reagents were of analytical grade. Tetraglycerin monolaurate condensed ricinoleic acid ester (CR-310) was supplied by Sakamoto Yakuhin Kogyo (Osaka, Japan). Soybean oil (Wako Pure Chemical Ind.) and *Moringa oleifera* oil (Yamakei Co., Ltd., Osaka, Japan) were used as continuous-phase medium. *M. oleifera* oil was used because of its functional attributes toward human health and good amount of oleic acid (Table 1). The oil obtained from *Moringa* is also much cheap in comparison with other industrial food grade oils.

2.2. Preparation of W/O emulsions

W/O emulsions were prepared from a dispersed aqueous phase and a continuous oil phase. The dispersed phase contained (5–30 g 100 g^{-1}) ascorbic acid and (1 g 100 g^{-1}) magnesium sulfate. The magnesium sulfate was added to the dispersed phase to balance the ion–ion interaction and these magnesium ions acts as counter ions to stabilize the ascorbic acid by ion shielding effect as previously described by Lee et al. (2004). Soybean oil or *Moringa* oil, each containing (5 g 100 g^{-1}) CR-310 as emulsifier, was used as continuous phase separately. The weight fraction of the dispersed aqueous phase (ϕ_d) was fixed to 30 g 100 g^{-1}. Thirty milliliters of dispersed phase were added to seventy milliliters of continuous phase at room temperature (~25 °C), followed by emulsification at various rotation speeds for 5 min with a polytron homogenizer (PT-3000 Kinematica-AG, Littace, Switzerland).

2.3. Determination of average droplet diameter

For analysis of prepared W/O emulsions, microscopic observations were made with an optical microscope (DM IRM, Leica Microsystems, Wetzler, Germany). From these micrographs droplet size was measured and expressed as average droplet diameter (d_{av}). To determine d_{av}, the diameters of 250 droplets were measured with an image processing software (WinRoof ver. 5.6, Mitani Co., Fukui, Japan). Coefficient of variation (CV) was used as an indicator of droplet size distribution of the prepared emulsion. The CV (%) was defined as follows:

$$CV = (\sigma/d_{av}) \times 100 \quad (1)$$

where σ is the standard deviation of the droplet diameter (μm).

2.4. Measurement of fluid properties

Viscosity, density, and interfacial tension between the dispersed and continuous phases were measured at 25 °C. Viscosity was measured with a vibro viscometer (SV-10, A&D Co., Ltd., Tokyo, Japan). Density was measured with a density meter (DA-130 N, Kyoto Electronics Manufacturing Co., Ltd., Kyoto, Japan). The interfacial tension was measured with a Fully Automatic Interfacial Tensiometer (PD-W, Kyowa Interface Sciences Co., Ltd., Saitama, Japan) using a pendant drop method.

2.5. Physical stability of W/O emulsions

Stability studies were carried out either at 4 ± 0.1 °C in refrigerator or 25 ± 0.1 °C in incubator at 75% relative humidity. The

Table 1
Fatty acid composition of soybean and *Moringa* oil (Rahman et al., 2009; Wang, 2011).

Fatty acid profile	Range (g 100 g^{-1})	
	Soybean oil	*Moringa oleifera* oil
Myristic (14:0)	0.1	0–0.13
Palmitic (16:0)	11	5.57–9.26
Palmitoleic (16:1)	0.1	1–3.7
Stearic (18:0)	4	2.27–8.3
Oleic (18:1)	23.4	67.7–76
Linoleic (18:2)	53.2	0.27–1.29
Linolenic (18:3)	7.8	0.18–0.45
Arachidic (20:0)	0.3	1.98–4.7
Gadoleic (20:1)	–	1.2–2.7
Behenic (22:0)	0.1	3.72–7.4
Total PUFA[a]	57.87	0.4–1.4

[a] Total polyunsaturated fatty acid.

stability test was carried out for 30 days, and emulsions containing 10, 20 and 30 g 100 g^{-1} L-ascorbic acid were analyzed with regard to droplet size, droplet size distribution, color, and physical state. The droplet size was measured using image processing software, as described in Sect. 2.4. Color and physical state were observed qualitatively (Akhtar et al., 2010).

2.6. Retention kinetics of L-ascorbic acid in W/O emulsions

The concentration of vitamin C in W/O emulsions was determined according to 2,6-dichlorophenol indophenol titrimetric method No. 967.21 of AOAC (2003) with slight modification. Briefly 1 g of W/O emulsion was mixed with 10 mL of ethanol, and then treated by sonication for 20 min, followed by centrifugation for 3 min at 3000 rpm. One milliliter of supernatant was used to measure L-ascorbic acid concentration. All experiments were repeated in triplicate and mean value was calculated. The retention rate (r) calculated by using Eq. (2) was defined by the ratio of measured L-ascorbic acid concentration (C_{AA}) to initial vitamin C concentration ($C_{AA,0}$).

$$r = (C_{AA}/C_{AA,0}) \times 100 \quad (2)$$

2.7. Statistical analysis

Analysis of variance (ANOVA) tests were used to analyze the characterization data at a confidence level of 95%. Least significant difference (LSD) test with 95% confidence level ($P < 0.05$) was used to compare d_{av} at different homogenization speed and L-ascorbic acid concentration in the dispersed phase. The LSD was calculated using Statistix 8.1 software (Tallahassee, USA) and according to the method described by Steel, Torrie, and Dickey (1997).

3. Results and discussion

3.1. Effect of homogenization speed on droplet size of W/O emulsions

Fig. 2a shows the effect of homogenization speed on d_{av} and CV of the prepared W/O emulsions containing 10 g 100 g^{-1} L-ascorbic acid. W/O emulsions were successfully prepared at 5000 rpm or higher, whereas lowering homogenization speed resulted in incomplete homogenization with visible phase separation. d_{av} of the W/O emulsions prepared gradually decreased from 3.0 to 2.0 μm by increasing the homogenization speed. There was a significant difference ($P < 0.05$) in their d_{av} with increasing homogenization speed. The CV of the prepared W/O emulsions ranged between 13% and 22% with polydispersity.

Homogenization at 15,000 rpm increased the system temperature to more than 70 °C (Fig. 2b), leading toward reduction in L-ascorbic acid retention to 82–85 g 100 g^{-1} in prepared W/O emulsions (Fig. 2c) which agrees well with results of Lee et al. (2004). The CV of W/O emulsions at different temperatures remains in range from 14 to 18%. The increase in temperature has no direct relation with average droplet diameter, but has direct relationship with amount of L-ascorbic acid retained in W/O emulsions as depicted in Fig. 2b. The production of emulsion droplets requires high energy input. The droplet size of an emulsion produced by rotor–stator homogenization process depends on the rotational speed which represents the energy of homogenization. The higher the energy applied in the homogenization of emulsion, the smaller the droplet size produced. However, uniformity of droplet size distribution depended on the completion of droplet size reduction which required more energy (Affandi, Julianto, & Majeed, 2011). W/O emulsions prepared at 7000 rpm incorporated mild heat (42 °C) in the system and showed better L-ascorbic acid retention rate >95 g 100 g^{-1}. This homogenization speed was considered adequate and therefore applied to prepare W/O emulsions in later sections. Increasing the homogenization speed results in incorporation of heat in emulsion system, and this heat might result in destruction of L-ascorbic acid (Lee et al., 2004).

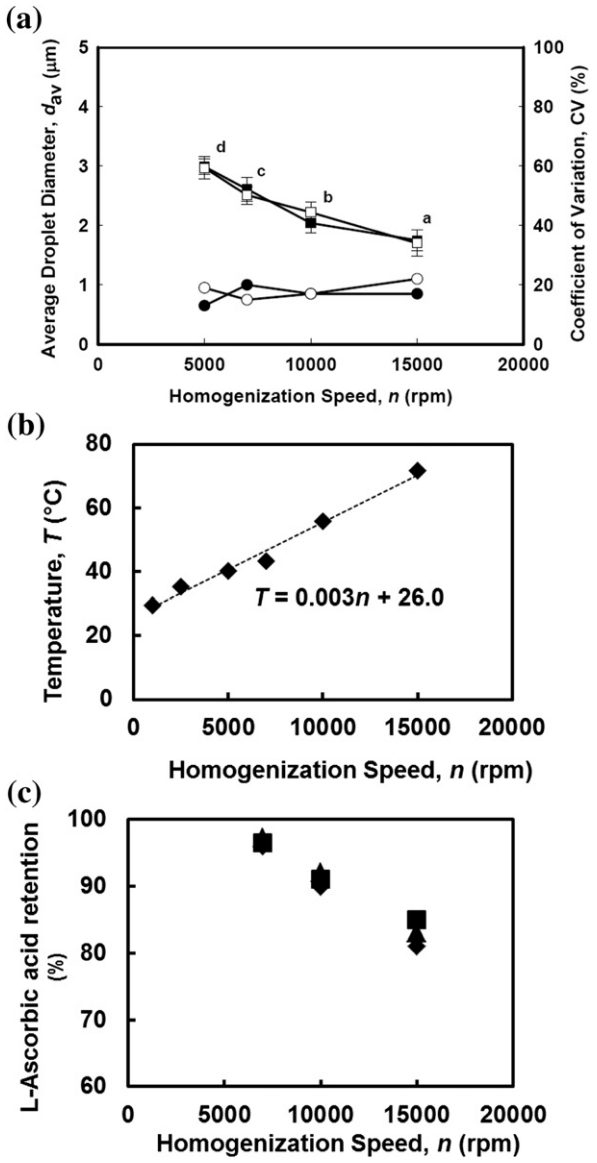

Fig. 2. (a) Effect of homogenization speed on average droplet diameter (d_{av}) and coefficient of variation (CV) of W/O emulsions containing 10 g 100 g^{-1} L-ascorbic acid. (■) represents soybean oil and (□) represents *Moringa* oil. Different letters (a, b, c, d) represent W/O emulsions (soybean and *Moringa* oil) have significantly difference in d_{av} at the 95% confidence level ($P = 0.05$). The symbol (●), (○) indicates CV of soybean oil and *Moringa* oil. (b) Effect of homogenization speed on temperature of prepared W/O emulsions. The fitted line had a coefficient of determination (R^2) of >0.98. (c) Effect of homogenization speed on L-ascorbic acid retention in prepared W/O emulsions. (◆) represents 10 g 100 g^{-1}, (■) represents 20 g 100 g^{-1} AA and (▲) represents 30 g 100 g^{-1} AA. AA stands for L-ascorbic acid.

3.2. Effect of L-ascorbic acid concentration in dispersed phase

Fig. 3 shows the effect of L-ascorbic acid concentration in the dispersed phase on d_{av} and CV of prepared W/O emulsions. There was no significant difference ($P > 0.05$) in their d_{av} with increasing

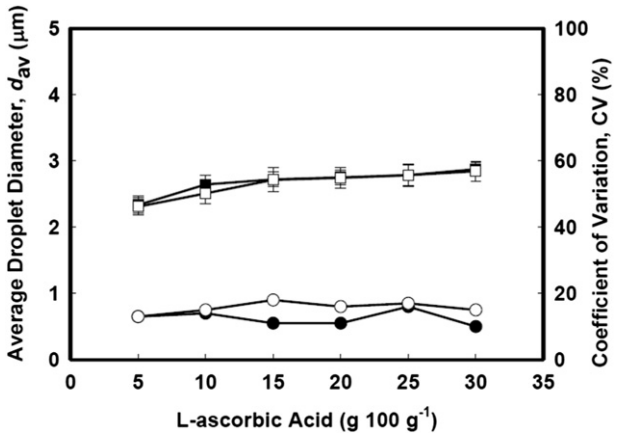

Fig. 3. Effect of different concentration of L-ascorbic acid on average droplet diameter (d_{av}) and coefficient of variation (CV) of W/O emulsions prepared at a homogenization speed of 7000 rpm (■) indicates d_{av} of soybean oil and (□) indicates *Moringa* oil. The symbol (●), (○) indicates CV of soybean oil and *Moringa* oil.

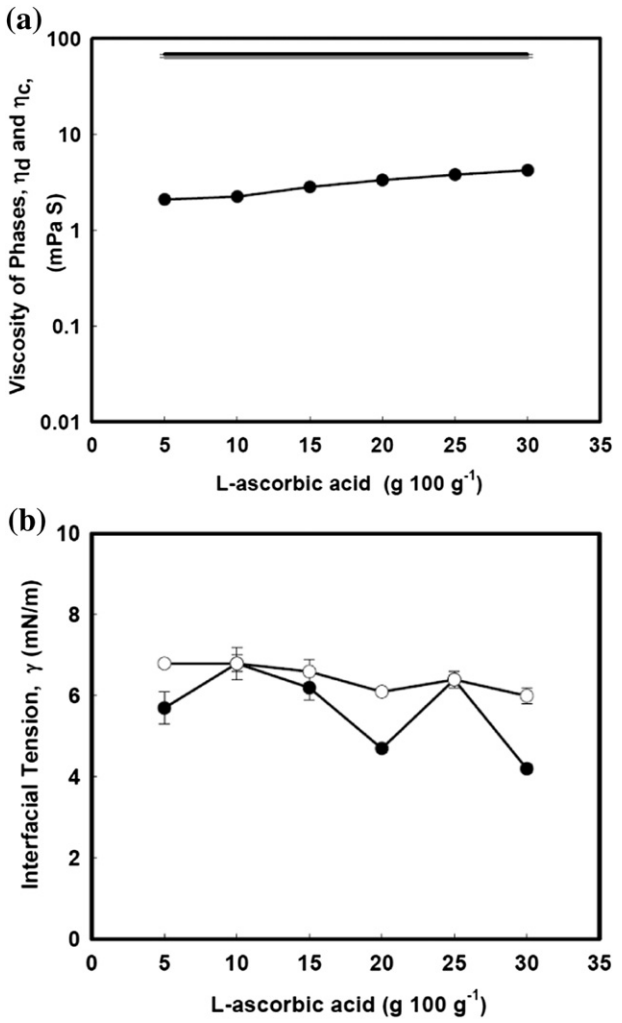

Fig. 4. (a) Variation of viscosity of the dispersed phase and continuous phase as a function of L-ascorbic acid concentration in the dispersed phase. η_d is the viscosity of dispersed phase (●), η_c is the viscosity of continuous phases (black line *Moringa* oil and gray line soybean oil) (b) Variation of the interfacial tension (γ) for soybean oil (●) and *Moringa* oil (○) as a function of L-ascorbic acid concentration.

L-ascorbic acid concentration from 5 g 100 g^{-1} to 30 g 100 g^{-1} in W/O emulsions. The oil type also did not affect droplet size of the prepared W/O emulsions. Both the soybean and *M. oleifera* oil have well balanced fatty acid profile (Table 1) with good amount of tocopherols. Their d_{av} ranged from 2.3 to 3.1 μm and slowly increased with increasing L-ascorbic acid concentration. Akhtar et al. (2010) reported that the oil droplet size of W/O/W emulsions containing 1 g 100 g^{-1} L-ascorbic acid ranged between 14.8 and 17.0 μm (Gallarate et al., 1999) also reported that the oil droplet size of different W/O/W emulsions containing 1 g 100 g^{-1} L-ascorbic acid varied from 15.6 to 42.2 μm. These previous studies regarding W/O/W emulsions containing L-ascorbic acid did not mention their internal aqueous droplet size. Major factors that affect d_{av} of emulsions are emulsion composition including the amount of emulsifier, and processing conditions. They play a vital role in determination of emulsions stability (Akhtar et al., 2010; Farahmand et al., 2006; Lee et al., 2004).

Viscosity (η), viscosity ratio (ζ) and interfacial tension (γ) are key fluid properties to investigate the droplet size of any emulsion. The soybean oil (η_c) had viscosity of 63.7 mPa s, while *Moringa* oil (η_c) had slightly higher viscosity of 68.7 mPa s. The gradual increase in η_d caused the slow increase in d_{av} of the prepared W/O emulsions (Fig. 4). γ between the soybean oil containing CR-310 and Milli-Q water containing different concentration of L-ascorbic acid (5–30 g 100 g^{-1}) was in between 4.5 and 6.3 mN/m (Fig. 4b),

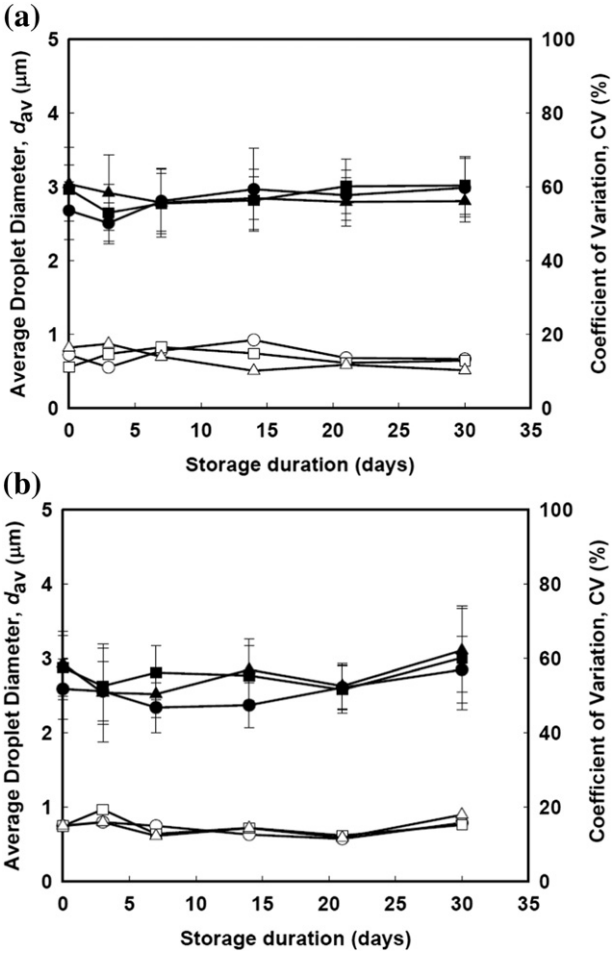

Fig. 5. Stability of W/O emulsions at 4 °C. (a) Soybean oil. (b) *Moringa* oil. The symbols (●) indicate 10 g 100 g^{-1} L-ascorbic acid (AA), (■) 20 g 100 g^{-1} AA, and (▲) 30 g 100 g^{-1} AA. The symbols (○) indicate CV of 10 g 100 g^{-1} AA, (□) 20 g 100 g^{-1} AA and (Δ) indicates 30 g 100 g^{-1} AA.

whereas it varied between 5.7 and 6.5 mN/m with increasing L-ascorbic acid concentration in *Moringa* oil. The decrease in interfacial tension by hydrophobic emulsifier (CR-310) improves the stability of oil/water interface, that results in stable formation of W/O emulsions (Scamehorn, 1986).

3.3. Physical stability of W/O emulsions containing L-ascorbic acid at 4 and 25 °C

Preparation of food-grade W/O emulsions containing vegetable oils (e.g., soybean oil and canola oil) is a challenging subject due to stability problems (Akhtar et al., 2010). The emulsion stability is defined in terms of physical and chemical stability of the emulsion.

Fig. 5 shows the stability of W/O emulsions containing 10–30 g 100 mL^{-1} L-ascorbic acid in the dispersed phase upon storage at 4 °C. There was little variation in their droplet size over 30 days of storage period, and their d_{av} remained in the range of 2.3–3.0 μm either in case of *Moringa* oil or soybean oil. The oil type did not affect the resultant droplet size, regardless of L-ascorbic acid concentration. All the W/O emulsions stored at 4 °C remained stable without any phase separation for more than 30 days. Fig. 6 depicts the stability of W/O emulsions containing 10–30 g 100 mL^{-1} L-ascorbic acid in the dispersed phase stored at 25 °C. Slight increases in d_{av} were seen in all the emulsions upon storage. The W/O emulsions stored at 25 °C had d_{av} of 2.5–3.4 μm for the soybean oil-containing systems and of 2.6–2.9 μm for the *Moringa* oil-containing systems during 30 days of storage period. All emulsions remained stable over 30 days, followed by a formation of thin aqueous layer at the bottom. Slight increase in CV up to 20% at 4 °C and 25 °C was seen in W/O emulsions containing *Moringa* oil. As for W/O emulsions containing soybean oil, their CV varied between 15 and 17% at 4 °C and 25 °C, respectively.

Organoleptic evaluation plays an important role in checking the physical stability of any emulsion (Akhtar et al., 2010). As for organoleptic evaluation, we qualitatively observed color, physical appearance and physical state of prepared W/O emulsions. The emulsions stored at 4 °C maintained whitish color for more than 30 days of storage, while those stored at 25 °C became first a lightly yellowish color after 17 days, followed by yellowish color that indicated destabilization of L-ascorbic acid in the emulsions. As for physical appearance, the W/O emulsions containing soybean oil had flow-like consistency, regardless of storage temperature. By contrast, the W/O emulsions containing *Moringa* oil had solid like consistency at 4 °C and flow like consistency at 25 °C. The chemical

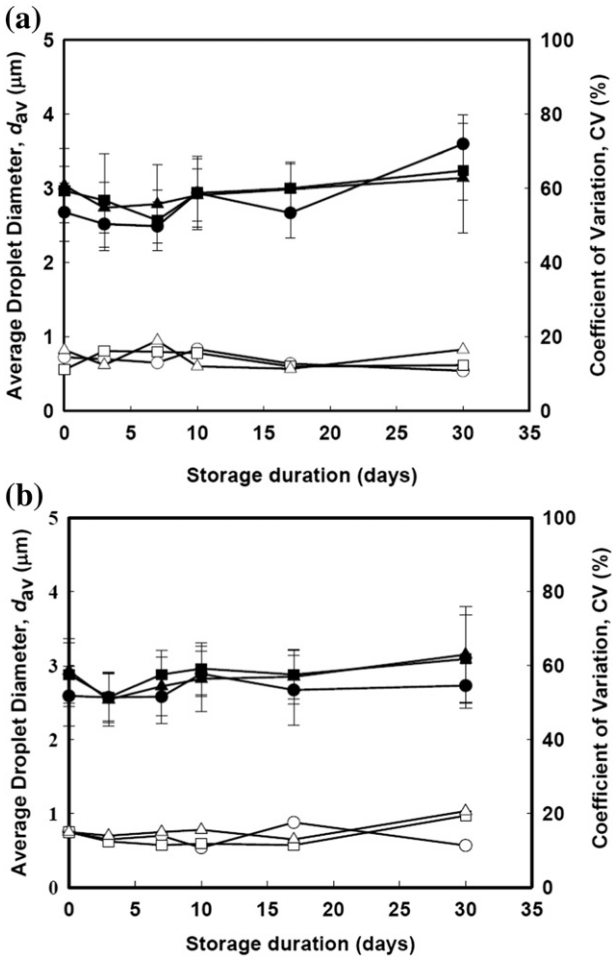

Fig. 6. Stability of W/O emulsions at 25 °C. (a) Soybean oil. (b) *Moringa* oil. The symbols (●) indicate 10 g 100 g^{-1} L-ascorbic acid (AA), (■) 20 g 100 g^{-1} AA, and (▲) 30 g 100 g^{-1} AA. The symbols (○) indicate CV of 10 g 100 g^{-1} AA, (□) 20 g 100 g^{-1} AA and (Δ) indicates 30 g 100 g^{-1} AA.

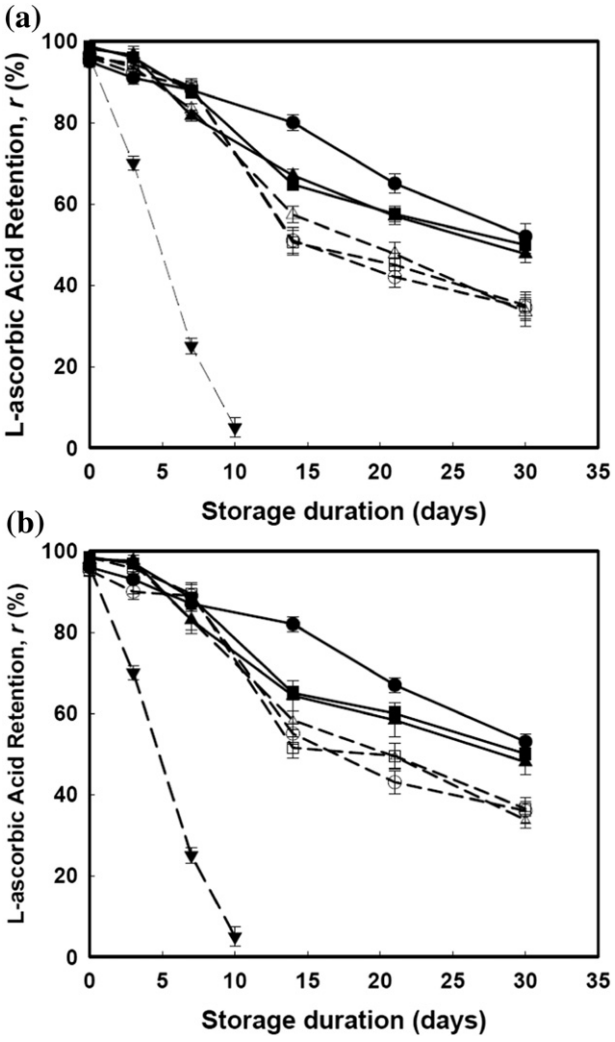

Fig. 7. (a) L-ascorbic acid retention in W/O emulsions containing soybean oil stored at 4 °C and 25 °C (b) L-ascorbic acid retention in W/O emulsions containing *Moringa* oil stored at 4 °C and 25 °C. The symbols (●) indicate 10 g 100 g^{-1} L-ascorbic acid (AA), (■) 20 g 100 g^{-1} AA, and (▲) 30 g 100 g^{-1} AA in W/O emulsions at 4 °C and the symbols (○) indicate 10 g 100 g^{-1} AA, (□) 20 g 100 g^{-1} AA and (Δ) indicates 30 g 100 g^{-1} AA in W/O emulsions at 25 °C. The symbol (▼) indicates L-ascorbic acid retention in Milli-Q water stored at 25 °C.

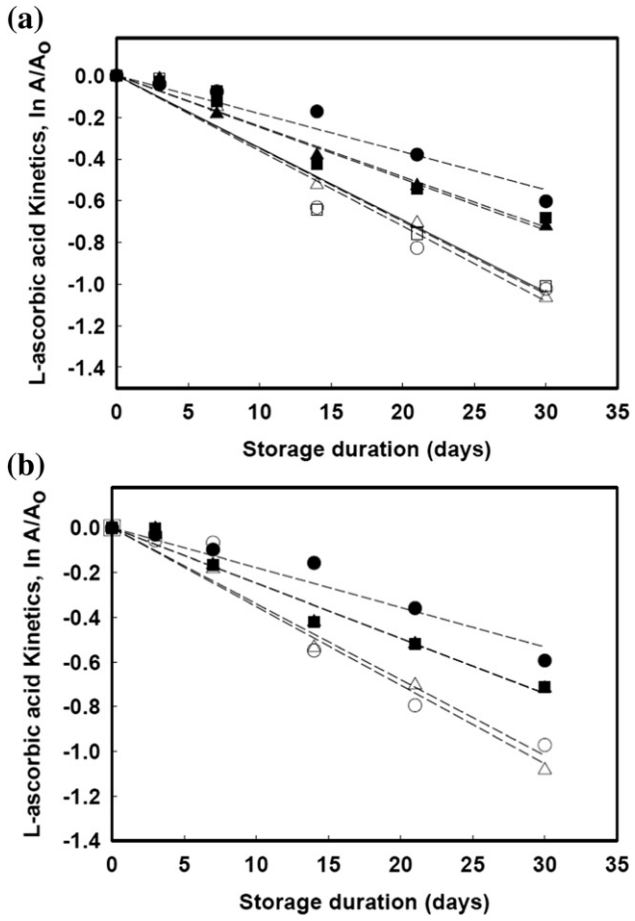

Fig. 8. (a) Retention kinetics of W/O emulsions containing soybean oil stored at 4 °C and 25 °C. Rate constants, coefficients of determination, and half-life are presented in Table 2. (b) Retention kinetics of W/O emulsions containing *Moringa* oil stored at 4 °C and 25 °C. Rate constants, coefficients of determination, and half-life are presented in Table 2. The symbols (●) indicate 10 g 100 g^{-1} L-ascorbic acid (AA), (■) 20 g 100 g^{-1} AA, and (▲) 30 g 100 g^{-1} AA in W/O emulsions at 4 °C and the symbols (○) indicate 10 g 100 g^{-1} AA, (□) 20 g 100 g^{-1} AA and (Δ) indicates 30 g 100 g^{-1} AA in W/O emulsions at 25 °C.

stability of L-ascorbic acid encapsulated in the W/O emulsions prepared in this study will be described in the next section.

3.4. Retention kinetics of W/O emulsions containing L-ascorbic acid

The chemical stability of L-ascorbic acid encapsulated in emulsions and creams has been studied in emulsions and creams by several research groups (Elmore, 2005; Farahmand et al., 2006; Gallarate et al., 1999; Lee et al., 2004; Raschke et al., 2004). L-ascorbic acid and its derivatives have been used in a variety of food products and cosmetic formulations as an antioxidant, pH adjuster, anti-aging and photoprotectant (Elmore, 2005). The control of instability of L-ascorbic acid poses a significant challenge in the development of cosmetic and food-grade formulations. Fig. 7 shows L-ascorbic acid retention in W/O emulsions stored at 4 °C. The prepared W/O emulsions containing 10–30 g 100 g^{-1} L-ascorbic acid exhibited 50 g 100 g^{-1} retention of L-ascorbic acid after 30 days of storage. There was no significant difference ($P > 0.05$) between two different continuous phases, as they hardly affected L-ascorbic acid retention after each storage period. By contrast, the W/O emulsions containing L-ascorbic acid stored at 25 °C exhibited 30 g 100 g^{-1} retention of L-ascorbic acid after 30 days storage (Fig. 7). The d_{av} was in range from 2.6 to 3.4 μm at 4 °C and 25 °C respectively, during retention kinetics evaluation. The temperature had a major effect on L-ascorbic acid degradation (Yuan & Chen, 1998). The observed retention rates were in accordance with a previous study by (Lee et al., 2004). Those authors concluded that encapsulation of L-ascorbic acid in W/O/W emulsions had beneficial effect on release profile of L-ascorbic acid. Ahmad et al. (2011) demonstrated that rate of oxidative degradation in L-ascorbic acid cream formulation in the dark was about 70 times slower than that in presence of light. L-ascorbic acid retention in Milli-Q water at 25 °C is also presented in Fig. 7. The L-ascorbic acid retention in W/O emulsions was higher than that in Milli-Q water during their storage. Rapid ionization in aqueous solution results in rapid loss of L-ascorbic acid (Matei, Soceanu, Dobrinas, & Magearu, 2009; Yuan & Chen, 1998).

Fig. 8 shows the retention kinetics of L-ascorbic acid in W/O emulsions during storage. In (A/A_0) represents the fraction of remaining L-ascorbic acid in the emulsions, where A is the L-ascorbic acid concentration after storage and A_0 is the initial L-ascorbic acid concentration. The results indicate that the L-ascorbic acid retention follow first order kinetics with respect to time. The retention of L-ascorbic acid as first-order kinetics was also reported by different research groups (Watanabe, Suzuki, Nakanishi, Sakuragochi, & Adachi, 2012). The retention kinetics of L-ascorbic acid in W/O emulsions stored at 4 and 25 °C was also evaluated by the following first-order kinetics equation:

$$A/A_0 = \exp(-kt) \quad (3)$$

where k is the rate constant and t is the storage time. The rate constant of the first-order kinetics was calculated to best-fit the experimental results by the regression function of Microsoft Excel 2007. The lines fitted by Eq. (2) are presented in Fig. 8. The calculated coefficients of determination (R^2) for all W/O emulsions were

Table 2
Rate constants and half-life of L-ascorbic acid degradation in different W/O emulsions.

Treatment	Rate equation	R^2	Rate constant k $(day)^{-1}$	Average value of rate constant k $(day)^{-1}$	Half life (day) $t_{1/2} = 0.693/k$
4 °C (Soybean oil), 10 g 100 g^{-1}	$\ln(A/A_0) = -0.0183t$	0.95	0.018	0.022	31.5
4 °C (Soybean oil), 20 g 100 g^{-1}	$\ln(A/A_0) = -0.0242t$	0.96	0.024		
4 °C (Soybean oil), 30 g 100 g^{-1}	$\ln(A/A_0) = -0.0249t$	0.98	0.025		
25 °C (Soybean oil), 10 g 100 g^{-1}	$\ln(A/A_0) = -0.0361t$	0.94	0.036	0.035	19.8
25 °C (Soybean oil), 20 g 100 g^{-1}	$\ln(A/A_0) = -0.0350t$	0.94	0.035		
25 °C (Soybean oil), 30 g 100 g^{-1}	$\ln(A/A_0) = -0.0347t$	0.98	0.035		
4 °C (*Moringa* oil), 10 g 100 g^{-1}	$\ln(A/A_0) = -0.0178t$	0.95	0.018	0.023	30.1
4 °C (*Moringa* oil), 20 g 100 g^{-1}	$\ln(A/A_0) = -0.0247t$	0.97	0.025		
4 °C (*Moringa* oil), 30 g 100 g^{-1}	$\ln(A/A_0) = -0.0247t$	0.97	0.025		
25 °C (*Moringa* oil), 10 g 100 g^{-1}	$\ln(A/A_0) = -0.034t$	0.95	0.034	0.035	19.8
25 °C (*Moringa* oil), 20 g 100 g^{-1}	$\ln(A/A_0) = -0.0346t$	0.98	0.035		
25 °C (*Moringa* oil), 30 g 100 g^{-1}	$\ln(A/A_0) = -0.0352t$	0.98	0.035		

>0.95, indicating that fitting application of the first-order kinetics is reasonable. Rate constants and half-life of L-ascorbic acid retention in different W/O emulsions are also presented in Table 2. There was no significant difference ($P > 0.05$) in the retention kinetics of two different continuous phases (soybean oil or *Moringa* oil). The observed half-life for W/O emulsions (soybean oil and *Moring*a oil) at 4 °C is >30 days and >19 days for emulsions at 25 °C respectively. The half-time values for the W/O emulsions stored at 4 °C show a better stability, compared to those stored at 25 °C.

The photolysis kinetics of L-ascorbic acid in cream formulations was investigated by Ahmad et al. (2011). They concluded that L-ascorbic acid retention follows first-order kinetics and this retention is affected by the concentration of active ingredients, pH, and viscosity of the medium. Watanabe et al. (2012) concluded that retention of catechins follows first-order kinetics in O/W emulsions containing L-ascorbic acid. More research is needed to completely elucidate the retention profile of L-ascorbic acid in W/O emulsions containing higher concentration of L-ascorbic acid.

4. Conclusions

Food-grade W/O emulsions containing L-ascorbic acid up to 30 w/v% in the dispersed aqueous phase were successfully prepared. Adequate homogenization conditions enabled preparing W/O emulsions with an L-ascorbic acid retention rate >95%, regardless of L-ascorbic acid concentration. The prepared W/O emulsions containing high concentration of L-ascorbic acid were well stabilized at 4 and 25 °C for more than 30 days with good organoleptic profile. All prepared W/O emulsions showed relatively higher retention of ~50 g 100 g^{-1} at 4 °C and ~30 g 100 g^{-1} at 25 °C after 30 days storage with first order kinetic fitting. These findings were not affected by the type of oil used in this study. The above retention time could be further improved by using mixed emulsifier system or addition of some bulking agents like gelatin that increase the viscosity of dispersed phase. The above results provide a foundation for better understanding of W/O emulsion preparation containing high concentration of vital vitamins such as L-ascorbic acid, thus having a practical implication in functional food development and for better application in product development science.

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