



# Carotenoid stability in high total solid spray dried emulsions with gum Arabic layered interface and trehalose–WPI composites as wall materials



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## ABSTRACT

The present study investigated the ability of spray dried single layer (SL) and layer-by-layer (LBL) high total solid emulsions with carbohydrate (trehalose) and non-carbohydrate (WPI) solids to stabilize carotenoids upon storage at 35 °C, 50 °C, and 65 °C. Carotenoid loss followed first order loss kinetics, and increased with increasing storage temperature. Rapid initial first order loss followed by a second, less rapid first order loss was observed. Storage of the systems above the  $T_g$  reduced carotenoid loss in the initial first order loss. The loss of carotenoids in LBL system was more temperature dependent initially but SL system was more temperature dependent in the second first order loss step. LBL system showed slower loss rate of carotenoids in the initial first order loss step and at 65 °C in the second step. Carotenoid retention was significantly higher in LBL system upon storage at 65 °C. *Industrial relevance:* Although layer-by-layer (LBL) technique has been known to produce emulsions with better stability towards environmental stresses, few have reported the application of LBL technique using systems with high total solids. The application of LBL technique on emulsion with high total solids and subsequent spray-drying of the emulsion in this manuscript will provide useful information to the food and pharmaceutical industries. The possibility to spray-dry such systems with high total solids producing high quality powders would be feasible to the industry as it greatly reduces production cost. The present study also reports on the carotenoid loss kinetics of dehydrated concentrated systems with carbohydrate and non-carbohydrate mixtures as wall materials and compares the ability of single layer (SL) and LBL systems in preventing the loss of the encapsulated carotenoids.

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## 1. Introduction

Studies have found that the application of layer-by-layer (LBL) interfacial structures in LBL emulsions can result in emulsions with higher stability towards environmental stresses such as heat treatment, variations in pH, freeze–thaw cycles, lipid oxidation and ionic strength (Aoki, Decker, & McClements, 2005; Gharsallaoui et al., 2010; Güzey & McClements, 2006; Lim, Griffin, & Roos, 2014; Ogawa, Decker, & McClements, 2003a). The higher stability of LBL system is due to the thicker and denser interfacial layer of the particles, higher steric repulsion, as well as lower van der Waals attraction strength (Benjamin, Silcock, Leus, & Everett, 2012; Gu, Decker, & McClements, 2005; Harnsilawat, Pongsawatmanit, & McClements, 2006; Moreau, Kim, Decker, & McClements, 2003). The thicker interfacial layer of LBL systems can increase the stability of the oil particles towards disruptions providing better protection towards the encapsulated materials (Güzey & McClements, 2006; McClements, 1999). LBL emulsion can be produced via electrostatic attraction between a charged primary layer with an oppositely charged secondary layer

present in the continuous phase (Lim & Roos, 2015; Moreau et al., 2003). Whey protein isolate (WPI) was used as the primary layer and gum Arabic as the secondary layer in this study. Due to its ability to be positively or negatively charged by changing the pH of the aqueous phase, protein is widely used as the primary layer in LBL systems (Gharsallaoui et al., 2010; Gu, Decker, & McClements, 2004; Klein, Aserin, Ishai, & Garti, 2010). The isoelectric point (pI) of β-lactoglobulin is 5.2 (Bryant & McClements, 1998) while α-lactalbumin is 4.1 (Weinbreck, de Vries, Schrooyen, & de Kruif, 2003). As gum Arabic has a pKa value of approximately 2.2, gum Arabic will be negatively charged above pH 2.2 (Weinbreck, Tromp, & de Kruif, 2004). Generally, the use of proteins as emulsifiers results in small oil droplets in emulsions with poor stability towards environmental stresses. On the other hand, polyelectrolytes produced oil droplets with better stability towards environmental stresses but were incapable of producing small oil droplets unless used in excess quantities (McClements, 2003).

Carotenoids are commonly found in fruits and vegetables with β-carotene, a highly lipophilic carotene having the highest vitamin A activity and rate of conversion to vitamin A among the provitamin A carotenoids (Grune et al., 2010). β-Carotene is also capable of showing antioxidant and anticancer properties, and may prevent heart diseases (Albanes, 1999; Bendich & Olson, 1989; Omenn et al., 1996).

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Degradation of  $\beta$ -carotene occurs mainly through oxidation (Simpson, 1985) and isomerization (Sweeney & Marsh, 1971). On the contrary, lutein, due to the presence of hydroxyl groups in its molecular structure is categorized as xanthophyll. Lutein is commonly found in green vegetables and can minimize light-induced skin damage (Ribaya-Mercado & Blumberg, 2004) as well as prevents cataracts and macular degeneration (Kachik, Bernstein, & Garland, 1997). Chronic and degenerative diseases in humans can be reduced with the consumption of food products consisting of bioactive compounds (van Dokkum, Frølich, Saltmarsh, & Gee, 2008). The low bioavailability of carotenoids as crystals or within protein complexes in fruits and vegetables reduces adsorption in the gastrointestinal tract during digestion (Williams, Boileau, & Erdman, 1998). Nonetheless, the use of carotenoids with oil can improve the bioavailability of carotenoids as oil increases carotenoid adsorption (van Het Hof, West, Weststrate, & Hautvast, 2000). Therefore, the incorporation of carotenoids in the lipid phase of an oil-in-water (O/W) emulsion can enhance its bioavailability. The lipid phase can be emulsified with an emulsifier containing an aqueous phase followed by the addition of glass forming hydrophilic component and dehydration of the emulsion to produce a continuous phase of the dried formulation (Drusch, Serfert, Van Den Heuvel, & Schwarz, 2006; Ramoneda, Ponce-Cevallos, Buera, & Elizalde, 2011; Spada, Noreña, Marczak, & Tessaro, 2012; Mehanna, Alwattar, & Elmaradny, 2015).

Microencapsulation utilizes wall materials to entrap core materials protecting it from environmental stresses to provide longer shelf life and enable controlled release of the core materials (Shahidi & Han, 1993). Microencapsulation is commonly achieved through spray drying in the food industry (Ré, 1998). Spray drying is also straightforward and economical giving high quality powders with a long shelf life. Maltodextrins, trehalose, milk proteins, corn syrup and modified starch are frequently used as wall materials in spray drying (Desobry, Netto, & Labuza, 1997; Drusch et al., 2006; Hogan, McNamee, O'Riordan, & O'Sullivan, 2001; Liang, Huang, Ma, Shoemaker, & Zhong, 2013; Mehanna et al., 2015; Shaw, McClements, & Decker, 2007). Crystallization of the wall materials in powders can lead to degradation of released carotenoids containing a lipid phase as a result of direct exposure to environmental stresses (Buera, Schebor, & Elizalde, 2005). Nevertheless, studies have shown that mixtures of sugars and proteins can delay the crystallization rate of the sugar component (Haque & Roos, 2004; Jouppila & Roos, 1994). Our earlier studies also showed that the application of LBL interfacial structures with trehalose, and the mixture of trehalose and maltodextrin (DE 10) as wall materials reduced the loss of carotenoids upon storage (Lim & Roos, 2016; Lim et al., 2014). The objectives of the present study were to obtain single layer (SL) and LBL powders by spray drying emulsions with high total solid content as well as to determine the ability of the SL and LBL powders having carbohydrate (trehalose) and non-carbohydrate (WPI) mixture as wall materials to protect carotenoids upon storage in the vicinity of the glass transition temperature ( $T_g$ ) of the carbohydrate. Data on carotenoid loss kinetics and the ability of SL and LBL systems in preventing the loss of encapsulated carotenoids in spray dried systems stored in closed containers were reported, providing important information to food and pharmaceutical ingredients and formulations. The application of concentrated systems having carbohydrate and non-carbohydrate mixture as wall materials for spray drying will be beneficial for the industries as there are few reports on the use of such a system.

## 2. Materials and methods

### 2.1. Materials

Sunflower oil was Musgrave Excellence™ (Spain), whey protein isolate (WPI, Isolac) was from Carbery Food Ingredients (Ballineen, Ireland), gum Arabic (Sigma Aldrich G9752) from Sigma Aldrich

(Stenheim, Germany), trehalose (crystalline dehydrate) from Hayashibara Shoji Inc. (Japan), all-trans- $\beta$ -carotene (crystalline Type I, synthetic, >93% (UV)) from Sigma-Aldrich (U.S.A.), and lutein (Marigold) from Shaanxi Sciphar Biotechnology Co. Ltd. (China). All other chemicals were purchased from Sigma-Aldrich, Inc. (Dublin, Ireland) and they were of analytical grade.

### 2.2. Emulsion preparation

The emulsions were prepared using methods modified from our earlier study (Lim & Roos, 2015, 2016). The emulsions prior to spray drying were SL and LBL which consisted of 18.2% sunflower oil and carotenoids, 18.18% trehalose, 9.09% WPI, and water (adjusted to pH 3.5 using citric acid) and with and without gum Arabic (0.27%), respectively. WPI was dispersed in deionized water (19.36%, w/w, in water) and the dispersion was allowed to hydrate for 2 h to enhance its hydration. The dispersion was adjusted to pH 3.5 using citric acid solution (10% w/w).  $\beta$ -Carotene (0.05%, w/w, of oil) and lutein (0.05%, w/w, of oil) were dissolved in sunflower oil and mixed with a Silverson mixer (Model AXR, Silverson Machines Ltd., Chesham, UK) at 50 °C. A Silverson mixer was used to mix the oil phase (2 parts of sunflower oil) and water phase (1 part of water used initially for the dispersion of WPI) at the minimum speed for 60 s to obtain pre-emulsion. The pre-emulsions were subsequently homogenized at room temperature using a two-stage valve homogenizer (APV-1000, APV Homogenizer Group, Wilmington, MA, USA) for 3 cycles at 240 bar (200 bars for the first stage and 40 bars for the second). A mixture of trehalose and WPI (ratio of 21:8) was used as wall material. Trehalose (46.41%, w/w) was dissolved in deionized water and citric acid solution mixture (40.21% water and 59.79% citric acid solution) using a Silverson mixer at 50  $\pm$  1 °C. WPI (30.77%, w/w) was dispersed in deionized water, stirred with a rod, and allowed to hydrate for 2 h to ensure complete hydration. The trehalose solution and WPI dispersion were then mixed together using the Silverson mixer and the pH was adjusted to pH 3.5 using citric acid solution. To obtain SL emulsion, the emulsion was mixed with the trehalose and WPI mixture at a ratio of 1:2.14 for 30 min. Gum Arabic (5.66%, w/w, in water) was dissolved in deionized water and stirred for 2 h. The gum Arabic solution was adjusted to pH 3.5 with citric acid solution. LBL emulsion was obtained by mixing the gum Arabic solution with the emulsion at a ratio of 1:6 at room temperature for 30 min using a Silverson mixer. The emulsion with gum Arabic as the secondary layer was then mixed with the trehalose and WPI mixture at a ratio of 1:1.83 for 30 min. The SL emulsion was added with a similar amount of water instead of gum Arabic solution to achieve the same final weight as the LBL emulsion.

### 2.3. Spray drying and sample packaging

The emulsions were dehydrated using a single stage Niro 25 spray dryer (GEA Niro Production Minor, Soborg, Denmark) with inlet and outlet temperatures set at 185 °C and 85 °C, respectively equipped with a rotating disk atomizer at 24,000 rpm. The powder solids consisted of 39.79% oil with carotenoids, 39.75% trehalose and 19.87% WPI as their main components and they were rapidly cooled to room temperature, sealed in plastic bags, and stored at room temperature to prevent water uptake and physico-chemical changes prior to analysis. The water contents after spray drying were less than 3% (Table 1) and water activities were  $0.14 \pm 0.01a_w$  for SL and  $0.14 \pm 0.01a_w$  for LBL powders, respectively. The powders (2 g) were then transferred into 10 mL clear glass vials (Schott, Müllheim, Germany). The samples in vials were hermetically sealed with a vacuum using a freeze-drier (Lyovac GT 2, Steris®, Hürth, Germany) and packaged in plastic pouches (PA/PE 90, Fispas, Leamore Warehouse, Dublin, Ireland) using a vacuum sealing system (Polar 80, Henkelman Vacuum Systems, Hentogenbosch, Netherlands) in duplicates. The vacuum in the packages represents isolation of the systems from surrounding atmosphere and served as an indicator for leakage

**Table 1**

Water content, particle density, occluded air, interstitial air, bulk density, tapped bulk density (100 times), surface oil, and average volume–surface diameter ( $d[4,3]$ ) of single layer (SL) and layer-by-layer (LBL) spray dried emulsions with trehalose and WPI mixture as wall materials.

	SL	LBL
Water content (g/100 g SNF)	2.74 ± 0.03	2.89 ± 0.02
Particle density (g/cm <sup>3</sup> )	0.98 ± 0.02	0.99 ± 0.03
Occluded air (cm <sup>3</sup> /100 g)	20.67 ± 1.29	18.51 ± 2.74
Interstitial air (cm <sup>3</sup> /100 g)	101.63 ± 1.29	98.79 ± 2.74
Bulk density (g/cm <sup>3</sup> )	0.33 ± 0.01	0.35 ± 0.01
Tapped density (g/cm <sup>3</sup> )	0.49 ± 0.01	0.50 ± 0.01
Surface oil (g/100 g)	6.90 ± 0.14	3.90 ± 0.04
D [4,3] (μm)	46.77 ± 0.17	48.23 ± 0.13

Values are means ( $n = 3$ ) ± SD.

that could have led to possible loss or uptake of water during storage. Differential scanning calorimetry (DSC) (Mettler Toledo 821E, Schwerzenbach, Switzerland) with liquid N<sub>2</sub> cooling was used to determine the glass transition temperature ( $T_g$ ) of the powder solids. Samples of powders were prepared in DSC aluminum pans (40 μl; Mettler Toledo, Schwerzenbach, Switzerland), hermetically sealed, and analyzed. STARe thermal analysis software, version 6.0 (Mettler Toledo, Schwerzenbach, Switzerland) was used to analyze the thermograms. The  $T_g$  for the powder solids with the mixture of trehalose and WPI as wall materials was found to be 50 ± 0.2 °C for SL powders and 51 ± 0.2 °C for LBL powders. Samples of powders (2 g) in vials were stored in the vicinity of the  $T_g$  in incubators (TS 8136, Termaks, Bergen, Norway) set at 35 ± 1 °C, 50 ± 1 °C, and 65 ± 1 °C.

#### 2.4. Colorimetry

The color values ( $L^*$ ,  $a^*$ , and  $b^*$ ) of SL and LBL systems were measured directly from glass vials upon storage at 35 °C, 50 °C, and 65 °C for up to 78 days of storage (13 time points) using a pre-calibrated colorimeter (Minolta, CR300 meter). The  $a^*$  and  $b^*$  ranged from –120 to 120 measuring the chromatic components of green to red values and blue to yellow values, respectively while the  $L^*$  value ranged from 0 to 100 and corresponded to the luminance or lightness component.

#### 2.5. Water activity

Water activity ( $a_w$ ) of SL and LBL systems stored at 35 °C, 50 °C, and 65 °C was determined at room temperature using an AquaLab Water Activity Meter CX-2 with an internal temperature control (Decagon Devices, Inc., Pullman WA., U.S.A.). The  $a_w$  of the systems was determined in plastic cups for up to 78 days of storage over 13 time points.

#### 2.6. Extraction method and HPLC analysis

SL and LBL powders (0.5 g) were reconstituted in 2 mL of deionized water (KB Scientific, Ireland) and vortexed (Scientific Industries Inc., G-560E, NY, USA) at room temperature for 30 s. This was followed by destabilization of the emulsion using a 3.9 mL methanol:ethylacetate (1:1, v/v) mixture acting as organic solvents for the carotenoids and vortexed for 30 s. The methanol:ethylacetate mixture contained 0.2% butylated hydroxyl toluene (BHT) as antioxidant to prevent the loss of carotenoids during the extraction process. The oil particles within the samples were saponified using 1 mL of saturated potassium hydroxide in methanol (2 M) and were vortexed for 30 s separating the mix into the saponized fraction (lipid carrier) and unsaponized fraction (β-carotene and lutein). To ensure complete dissolution of the carotenoids present in the organic phase, 1 mL of dichloromethane was added and the samples were vortexed for 30 s. Finally, 3.8 mL of hexane was added into the samples and vortexed for 30 s

to improve separation of the organic phase. The samples were then left to stand for 30 min in the dark. The organic phase was removed with a glass pipette, filtered (Minisart RC 15, Sartorius Stedim Biotech GmbH, Goettingen, Germany), and transferred into HPLC vials (Dionex No: 055427, 1.5 ml w/Slit Septum, Unas, USA). The samples were injected at a volume of 10 μL into the HPLC system. Dionex ICS3000 (Sunnyvale, CA, U.S.A.) with a dual pump (DP-1, Dionex, Sunnyvale, CA, U.S.A.), autosampler (AS-1, Dionex, Sunnyvale, CA, U.S.A.), and photodiode-array detector (PDA ICS Series, Dionex, Sunnyvale, CA, U.S.A.) was used to quantify the amount of β-carotene and lutein. The column used in the study was a 250 mm × 4.6 mm i.d., 5 μm, reversed-phase Acclaim C30 analytical column alongside a 4 mm × 4 mm i.d. guard column of the same material (Dionex, Sunnyvale, CA, U.S.A.). An eluent system composed of acetonitrile (A), methanol:ethylacetate (1:1) (B) and 0.5% of 200 mmol acetic acid in water (C) was used with a gradient profile of 84.5% A, 15% B, and 0.5% C at –5 to 2 min, gradient of A and B from 2 to 12 min to 64.5% A, 35% B, and 0.5% C, and return from 34 to 40 min to 84.5% A, 15% B, and 0.5% C. Relative amounts of β-carotene and lutein were derived from absorption peak areas performed at 450 nm. The study on the stability of β-carotene and lutein was carried out for up to 78 days of storage (13 time points). Data of carotenoid degradation were fitted to first-order kinetics ( $\ln A/A_0 = -kt$ ) and the rate constants ( $k$ ) were derived from the slopes of linear regressions. Plot of  $\ln A/A_0$  against time (storage days) gave a straight line which justified the use of first order kinetics as carotenoid degradation is a typical first order reaction (Desobry et al., 1997; Hidalgo & Brandolini, 2008; Lim et al., 2014; Mahfoudhi & Hamdi, 2014). Activation energy was determined using the Arrhenius relationship  $k = Ae^{-E_a/(RT)}$  and plots of  $\ln k$  against  $T^{-1}$ .

#### 2.7. Optical microscope

The spray dried emulsions were dispersed in sunflower oil on a glass slide and images were captured using an optical microscope at 100× magnification (Olympus BX51) attached to a video camera (PixeLink A662, Ottawa, ON).

#### 2.8. Raman–FIB–SEM analysis

Raman–FIB–SEM analysis was done using RISE microscope; a unique integration of high resolution Confocal Raman Microscope (CRM) (WITec) with field-emission ultra-high resolution Scanning Electron Microscope (SEM) and Focused Ion Beam (FIB; TESCAN GAI3 GMU) allowing the combination of all devices into one chamber. The precision of about 2 μm of movement from FIB–SEM to the Raman position allowed straightforward identification of the exact place which was visualized under CRM and cut under the FIB–SEM. The powder was first visualized in SEM to locate a suitable position for FIB milling. Rough milling (750 pA) was performed in order to reduce the cutting time followed by the polishing procedure (approx. 100 pA). Then the powder was tilted to the position where the revealed surface was horizontal. After the SEM image was captured, the powder was moved to the Raman position (approx. movement of 180 mm). Under CRM, the position for Raman imaging was selected on the base of the white light image. Raman mapping was performed with 532 nm green laser with pixel size of 100 × 100 nm<sup>2</sup> and an integration time of 0.15 s. Post processing and visualization were completed in Project Plus software (WITec).

#### 2.9. Particle size, particle density, occluded air, interstitial air, bulk density, and surface oil

The particle size distribution of SL and LBL powders was determined by laser diffraction using Malvern Mastersizer 3000 with a powder feeder unit (Malvern, Worcestershire, UK). A helium gas Multivolume

Pycnometer 1350 (Micromeritics, Georgia, USA) was used to determine particle density. The occluded air and interstitial air of the spray dried emulsions were determined as per GEA Niro (2006). Tapped bulk density (100 taps) of the powders was determined with a tap volumeter (Copley, J. Engelsmann A.G., Ludwigshafen, Germany). Surface oil of the powders was determined by hexane washing. The powders (10 g) were washed for four times using 50 ml of hexane per wash. The hexane containing extracted fat residue was allowed to evaporate in a water bath set at  $50 \pm 1$  °C and transferred into an oven set at  $50 \pm 1$  °C until constant weight was obtained.

### 2.10. Powder flow testing

A Brookfield Powder Flow Tester (PFT) (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) was used to analyze flowability and bulk density of the powders. Axial and torsional speed of 1.0 mm/s and 1 rev/h, respectively was used. An aluminum trough with a perforated sheet of the annular shear cell was filled with powders, leveled using a curved profiled shaping blade, and the weight of powders was recorded before testing. Then, vane profiled lids with simulated 2B finish for the flat lid was attached to a compression plate. Instantaneous flow function test was used to measure flowability of the powders. Five uniaxial normal stresses of between 0.2 and 4.8 kPa were applied and three over consolidation stresses at each normal stress.

### 2.11. Emulsion characterization

Particle size ( $\zeta$ -average diameter), using dynamic light scattering, measurement range: 0.3 nm–10  $\mu$ m) of the reconstituted powders before and after 92 days of storage at 35 °C, 50 °C, and 65 °C was determined using a Zetasizer (Zetasizer Nano ZS, Malvern Instruments Ltd., Malvern, UK). The powders were reconstituted to the initial concentration (45% total solid content) using deionized water adjusted to pH 3.5 with citric acid solution. The emulsions were then diluted with deionized water (pH 3.5) at a ratio of 1:500 for the measurements of  $\zeta$ -average diameter. Lastly, the diluted samples were vortexed for 30 s to ensure homogeneity. Plastic cuvettes (square cuvettes, PS, 10 mm  $\times$  10 mm  $\times$  45 mm, SARSTEDT AG & Co, Nümbrecht, Germany) were used to determine the particle size with 1 ml of diluted emulsions. Measurements were completed at room temperature.

### 2.12. Statistical analysis

The analyses were completed in triplicates and results were expressed as mean  $\pm$  standard deviation. The carotenoid stability study was carried out in replicates with duplicated injections.

## 3. Results and discussions

### 3.1. Powder characteristics

High total solid single layer (SL) and layer by layer (LBL) emulsions were successfully spray dried producing high quality powders with no visual differences between the powders. The powders obtained were dispersed in sunflower oil and images were captured under a light microscope at 100 $\times$  magnifications. Fig. 1 showed that the SL and LBL powders obtained were similar in shape and contained dark spots indicating the presence of air vacuoles. Images from the Raman–FIB–SEM (Fig. 2) also showed that both SL and LBL powder particles had spherical shapes with slight wrinkles and surface roughness. The surface of a cut particle is shown in Fig. 2. The water content, particle density, occluded air, interstitial air, bulk density, tapped bulk density, surface oil, and particle size of the SL and LBL powders obtained were shown in Table 1. The powders have very similar characteristics as there were no significant differences in the particle density, occluded air, interstitial air, bulk density and tapped bulk density. Although there were no significant differences in the particle density, occluded air, and interstitial air between the powders, the higher particle density in LBL powder while higher occluded air and interstitial air in SL powder showed a trend similar to the powders obtained in our earlier study (Lim & Roos, 2016). Gum Arabic attached electrostatically on the charged WPI–oil interface in LBL layered interface resulting in higher overall particle density. Overall particle density of the powders obtained was lower than those having only carbohydrates as wall materials (Desobry et al., 1997; Drusch et al., 2006) as the presence of protein reduced particle density (Moreau & Rosenberg, 1999). The water content in SL powder was significantly higher than that in LBL powder. This was due to the smaller particle size of SL powder that gave a higher total surface area for water sorption from the surroundings during cooling of the powders to room temperature. The occluded air was fairly low in both powders as high total solid emulsions were used for spray-drying. High occluded air and low bulk density of powders are the consequences of low total solid formulations used for drying (Mistry, 2002). The surface oil of the powders was found to be significantly higher in the SL system than that in LBL system. The application of LBL interfacial structure increases the stability of the oil particles towards disruption (Güzey & McClements, 2006) occurring during atomization increasing microencapsulation efficiency of the carotenoids containing oil. There was a significant difference in the  $d[4,3]$ , average volume–surface diameter between SL and LBL powders with LBL powders having bigger particle sizes. The presence of gum Arabic in the continuous phase of the LBL emulsion increased its viscosity which affected particle formation during atomization in the spray drier producing larger particles. The flowability and bulk density as a function of major principal consolidation stress (kPa) of the SL and LBL powders are shown in Fig. 3. Both the SL and

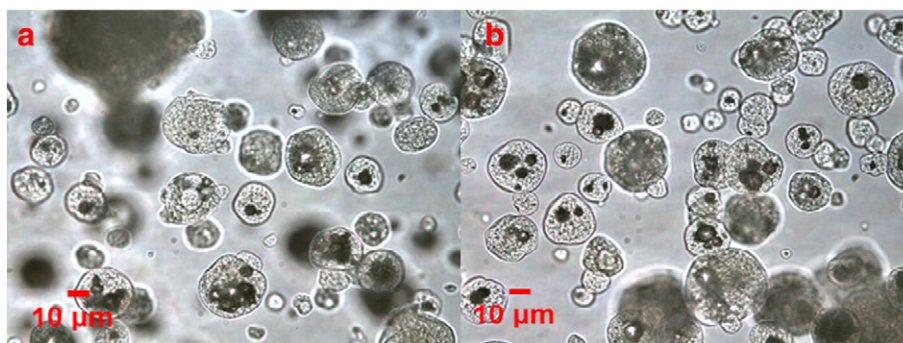
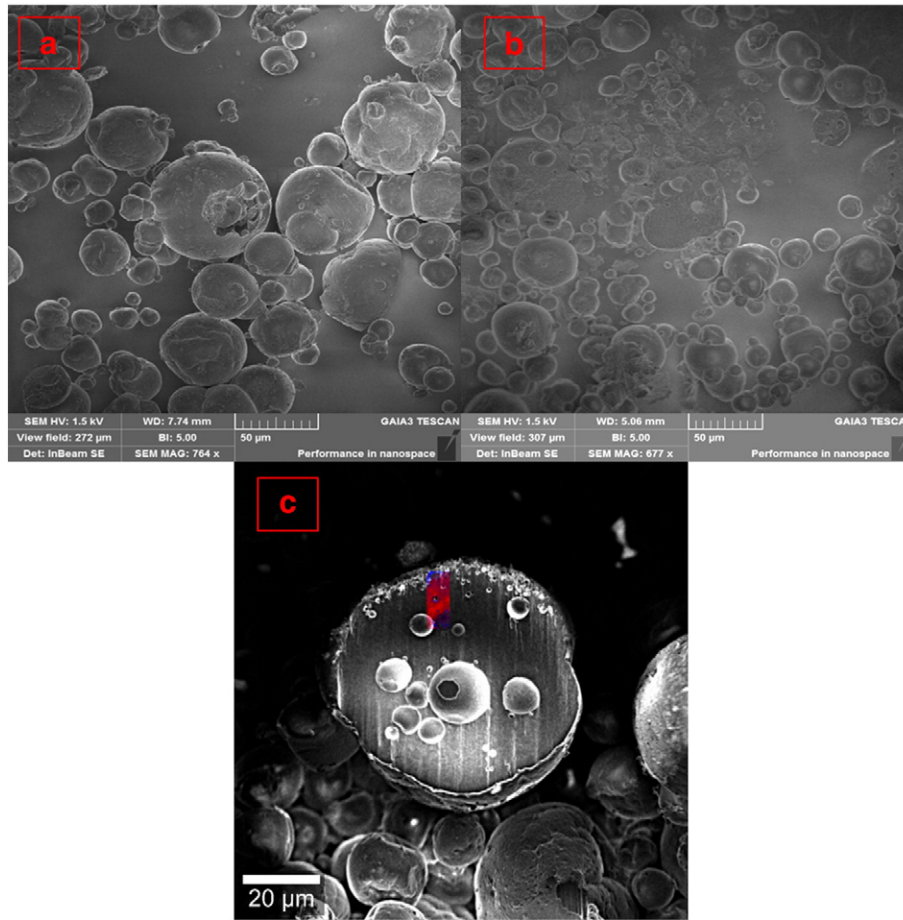


Fig. 1. Images from the light microscope at 100 $\times$  magnification of single layer (SL) (a) and layer-by-layer (LBL) (b) powders dispersed in sunflower oil.



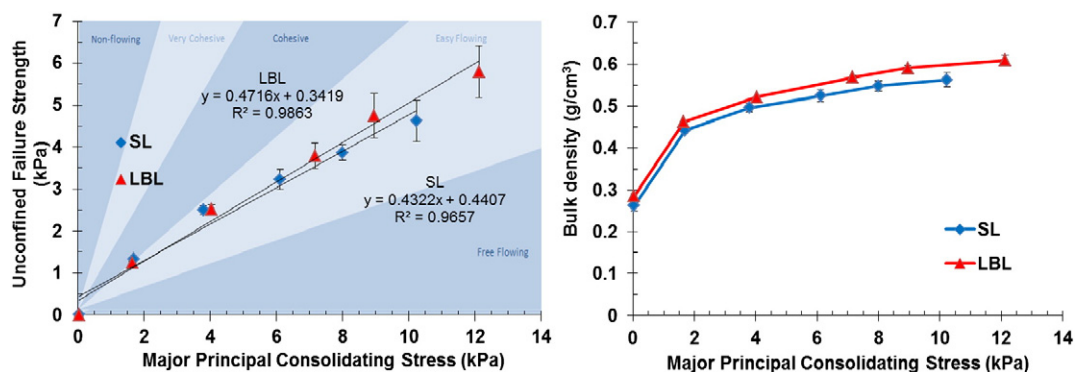
**Fig. 2.** Raman-FIB-SEM images showing single layer (SL) powder particles (a), layer-by-layer (LBL) powder particles (b), and a cut powder particle with the presence of lutein (red) in large quantities at interfaces and all-trans- $\beta$ -carotene (blue) dissolved in encapsulated oil and which appeared only in minor quantities at the cut surfaces (c).

LBL powders were generally ‘easy flowing’ with no significant differences in their flowability across the major principal consolidation stress (kPa). On the other hand, LBL powders have significantly higher bulk density across the major principal consolidation stress (kPa).

### 3.2. Color measurements, water activity, and $\zeta$ -average diameter

**Fig. 4** shows the color values ( $L^*$ ,  $a^*$ , and  $b^*$  values) of SL and LBL spray dried systems at 0 and after 78 days of storage at 35 °C, 50 °C, and 65 °C. There was no significant difference in the color values between SL and LBL systems at day 0. Generally, minor changes occurred

in the  $L^*$  (lightness) and  $a^*$  (redness) while bigger changes were observed in  $b^*$  (yellowness) values. The changes in  $L^*$  values were not significant except at 65 °C where  $L^*$  values of both SL (from 86.39 to 81.88) and LBL (from 86.29 to 83.18) powders decreased significantly. On the other hand, the  $a^*$  values of systems stored at 35 °C and 50 °C decreased significantly upon storage. This was in agreement with results obtained in other studies using systems with carotenoids upon storage due to carotenoid loss (Desobry et al., 1997; Mahfoudhi & Hamdi, 2014; Qian, Decker, Xiao, & McClements, 2012). However, minor changes with no significant differences in  $a^*$  values were observed in systems stored at 65 °C which corresponds to the  $L^*$  values where systems stored at



**Fig. 3.** Flowability showing unconfined failure strength (kPa) and bulk density as a function of major principal consolidation stress (kPa) of single layer (SL) and layer-by-layer (LBL) powders.

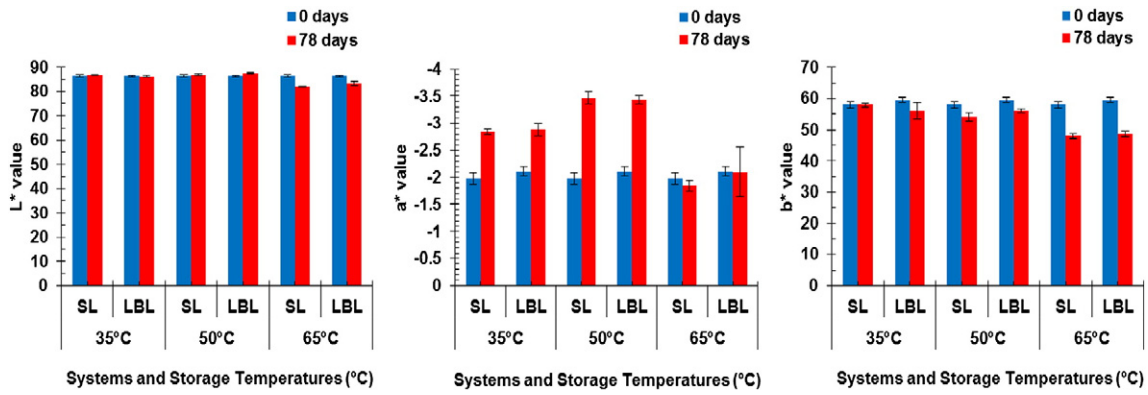


Fig. 4. L\* (lightness), a\* (redness), and b\* (yellowness) values of spray dried single layer (SL) and layer-by-layer (LBL) emulsions before and after storage for 78 days at 35 °C, 50 °C, and 65 °C.

65 °C showed a different trend compared to systems stored at 35 °C and 50 °C. Residual lactose present in WPI were capable of causing the Maillard reaction (non-enzymatic browning) that resulted in the changes of L\* and a\* values stored at 65 °C. Besides, lipid oxidation generates free radicals and reactive oxygen groups capable of attacking proteins and amino groups present in the continuous phase that results in browning (Pokorný, El-Zeany, Kolakowska, & Janiček, 1974; Potes, Kerry, & Roos, 2014; Zirlin & Karel, 1969). Generally, the b\* values reduced upon storage with significant difference observed in systems stored at 50 °C and 65 °C. However, several authors claimed that b\* value was not a good indicator of surface color changes in systems with β-carotene as the color range b\* value characterizes were not dominant (Elizalde, Herrera, & Buera, 2002; Mahfoudhi & Hamdi, 2014; Prado, Buera, & Elizalde, 2006; Spada et al., 2012).

Water activity ( $a_w$ ) of the SL and LBL systems before and after storage at 35 °C, 50 °C, and 65 °C for 78 days is shown in Fig. 5. The  $a_w$  of SL powders ( $0.14 \pm 0.01a_w$ ) and LBL powders ( $0.14 \pm 0.01a_w$ ) were similar to the  $a_w$  of powders frequently obtained from spray drying in the industry of around  $0.2a_w$  (Adhikari, Howes, Bhandari, & Langrish, 2009). Generally, there was no significant difference in the water activity before and after storage for 78 days at all temperatures in both SL and LBL systems. Vacuum was preserved in the sample packages and vials during the duration of the study and showed that no leakages occurred that could have affected the  $a_w$ . The effect of storage at 35 °C, 50 °C, and 65 °C for 92 days on ζ-average diameter in reconstituted SL and LBL powders is shown in Fig. 6. It can be seen initially at day 0 that the ζ-average diameter of SL oil particles was significantly smaller than those of LBL. The application of LBL interfacial structure increased the ζ-average diameter due to the presence of both WPI and gum

Arabic at the oil interface. There was no significant difference in the changes of ζ-average diameter of SL and LBL systems upon storage at 35 °C and 50 °C for 92 days. However, storage at 65 °C resulted in a significant increase of the ζ-average diameter in both SL and LBL systems. Kim, Decker, and McClements (2002) suggested that the denaturation of β-lactoglobulin resulted in widespread flocculation of oil droplets in O/W emulsions because of the increased surface hydrophobicity that increases the hydrophobic interaction between oil particles. Besides, protein sulfhydryl groups will be exposed due to protein denaturation increasing the disulfide bond between protein covered oil particles. β-Lactoglobulin and α-lactalbumin on the surface of oil particles will unfold upon heating above 65 °C as observed in DSC (Dalgleish, 1996).

3.3. Carotenoid stability

Quantitative values of carotenoid retention were determined using the peak areas of all-trans-β-carotene and lutein. First order kinetics was used to fit the data producing first order degradation kinetics of carotenoids in both SL and LBL systems. Straight lines obtained in the plots of  $\ln A/A_0$  against storage days (time) supported the application of first order kinetics. Many other authors also found that the degradation of carotenoids is a typical first order reaction (Achir, Randrianatoandro, Bohuon, Laffargue, & Avallone, 2010; Desobry, Netto, & Labuza, 1999; Henry, Catignani, & Schwartz, 1998; Mahfoudhi & Hamdi, 2014). Arrhenius plots and activation energies of all-trans-β-carotene and lutein for SL and LBL spray dried systems stored at 35 °C, 50 °C, and 65 °C for 78 days are shown in Fig. 7. Glass transition temperature ( $T_g$ ) of the systems too was presented in the figure. The HPLC chromatograms showed that the retention time of all-trans-β-carotene was  $32.5 \pm 0.1$  min and  $6.8 \pm 0.1$  min for lutein. The increase in isomers corresponds to the loss

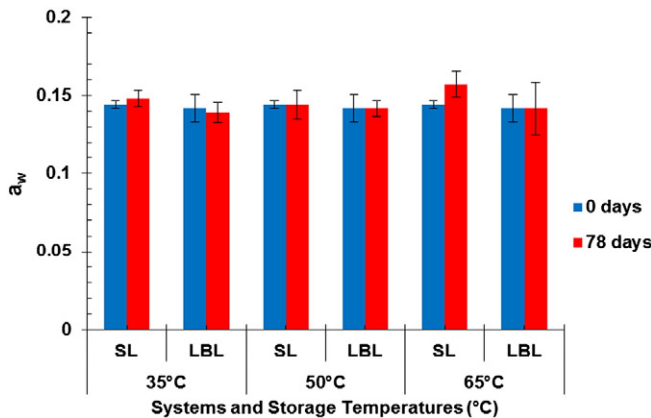


Fig. 5. Water activity ( $a_w$ ) of single layer (SL) and layer-by-layer (LBL) powders obtained by spray drying before and after storage at 35 °C, 50 °C, and 65 °C for 78 days.

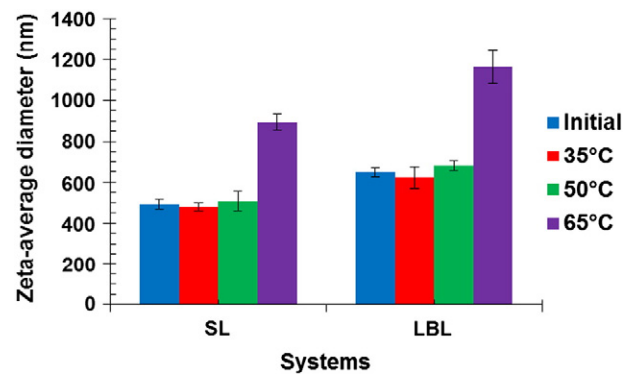


Fig. 6. Zeta-average diameter (nm) of oil particles in reconstituted single layer (SL) and layer-by-layer (LBL) powders before and after storage at 35 °C, 50 °C, and 65 °C for 92 days.

of all-trans- $\beta$ -carotene during heat treatment (Chandler & Schwartz, 1988). The main isomers observed in this study were the 15-cis- $\beta$ -carotene and 13-cis- $\beta$ -carotene. However, no observable trends can be obtained from the isomers as they fluctuate with time.

The presence of a two-step loss kinetics was noted in the loss of both all-trans- $\beta$ -carotene and lutein showing a fast initial first order loss kinetic followed by a second less rapid first order loss kinetic. This was in agreement with the observation made on the non-humidified spray dried system used in our earlier study (Lim & Roos, 2016). A similar observation was also made by Desobry et al. (1997) and Desobry et al. (1999) on the loss of  $\beta$ -carotene in a spray dried system. Nonetheless, a study by Drusch et al. (2006) also found a rapid initial increase of hydroperoxides followed by a second period of slower increase of hydroperoxides. The presence of surface oil on the powder surfaces increases the exposure of the carotenoid containing oil to the heat from surrounding as well as residual oxygen and oxygen present on the surface water. These exposures on the unprotected carotenoids containing oil led to the rapid initial loss of carotenoids. The higher amount of surface oil on SL powders led to the higher degradation rate of both all-trans- $\beta$ -carotene and lutein in SL system during the rapid initial loss. Besides, oxygen trapped within the glassy matrix of the powders is capable of causing oxidation leading to a rapid loss of carotenoids as oxidation is the main cause of carotenoid degradation (Qiu, Chen, & Li, 2009). Oxygen containing vacuoles were formed within the powders as a result of thermal expansion of trapped air bubbles upon hardening of the glass formers (Aguilera, 1990; Tewa-Tagne, Briançon, & Fessi, 2007). The activation energies of LBL system was higher than SL system in the initial first order loss indicating that the loss of carotenoids in LBL system was more temperature dependent. This was attributed to the presence of gum Arabic at the oil–WPI interface that reduced heat transfer towards the encapsulated carotenoids and effectively reducing carotenoids loss. Nonetheless, the ability of the LBL system in reducing heat transfer will reach a maximum limit with increasing storage temperature resulting in higher carotenoid loss. Likewise, the local  $T_g$  at the oil–WPI interface was increased by the presence of gum Arabic reducing flow or structural collapse at higher temperature. Therefore, LBL powders have lower density or hardness at the vicinity of the oil particles increasing the temperature dependence on the loss of carotenoids. However, in the second first order loss kinetic, the loss of carotenoids in SL system was more temperature dependent than LBL system. This showed that the SL system was less stable towards environmental stresses such as heat during long term storage. As structural collapse occurred at the rate of  $T - T_g$  (Levi & Karel, 1995), the presence of gum Arabic at the oil–WPI interface results in a harder and denser area around the oil particles over long term storage reducing the temperature dependence of carotenoid loss in LBL system.

The loss of both all-trans- $\beta$ -carotene and lutein in this study increased with increasing storage temperature and is a phenomenon commonly observed in other carotenoid containing studies (Caliskan,

Lim, & Roos, 2015; Desobry et al., 1997; Koca, Burdurlu, & Karadeniz, 2007; Hidalgo & Brandolini, 2008; Spada et al., 2012). Nonetheless, it was noted that the loss of carotenoids above the  $T_g$  was reduced based on the rate constants acquired in the initial rapid first order loss kinetics. This can be attributed to the lower solubility of oxygen with rising temperature of up to 100 °C (Hildebrand, 1952; Wilhelm, Battino, & Wilcock, 1977). The lower amount of oxygen dissolved in the surface water of the systems with higher storage temperatures led to the lesser amount of oxygen available to diffuse towards the carotenoid containing oil particles. This effectively reduced the loss of carotenoids in both SL and LBL powders. Besides, storing the systems above the  $T_g$  caused a dynamic process of structural collapse at a rate of  $T - T_g$  (Levi & Karel, 1995). Even though no physical collapse of the powders was observed, the increased molecular mobility and reduced viscosity above the  $T_g$  of the systems (Roos & Karel, 1991) lead to a thicker membrane on the powder particles. The resulting harder and denser powder particles effectively reduced carotenoid loss by reducing heat transfer from the surroundings towards the encapsulated carotenoid containing oil. However, during long term storage, a higher increase in the loss of all-trans- $\beta$ -carotene and lutein was observed above the  $T_g$  as seen in the second slower first order loss kinetics. The loss of carotenoids and increased isomerization gave higher loss rate constants above the  $T_g$  during long term storage as glass transition leads to the release of the encapsulated oil (Shimada, Roos, & Karel, 1991). The main factor for carotenoid loss is isomerization (Qiu et al., 2009) and is largely due to light and heat exposure. Free radicals generated from lipid oxidation over storage as well as reactive oxygen species are capable of reacting with the carotenoids present increasing the loss of carotenoids. Labuza, Heidelbaugh, Silver, and Karel (1971) stated that the rate of lipid oxidation increased in systems with  $a_w$  below 0.2 or above 0.5.

The loss of carotenoids in this present study was more rapid than those of the non-humidified spray dried powders from an earlier study (Lim & Roos, 2016) with a mixture of trehalose and maltodextrin (DE 10) as glass formers based on the rate constants. The mixture of carbohydrate (trehalose) and non-carbohydrate (WPI) as glass formers resulted in the higher porosity and lower density of the powders obtained. Density of spray dried powders was found to increase while the porosity decreased with increasing amounts of lactose in a WPI–lactose wall matrix system (Moreau & Rosenberg, 1999). A study by Carneiro, Tonon, Grosso, and Hubinger (2013) also found that powders having a mixture of maltodextrin and whey protein concentrate as wall materials have the lowest bulk density indicating a higher amount of occluded air. The higher porosity and lower density of the powders in this study allows greater heat permeability towards the encapsulated carotenoids that increased carotenoid loss. WPI preferably migrates to the particle surface (air–water interface) due to its high surface activity forming a protein rich layer (Adhikari et al., 2009; Wang, Jiang, & Zhou, 2013). Fäldt and Bergenstahl (1994) found that the surface-active protein predominates at the surface of spray dried carbohydrate–protein system.

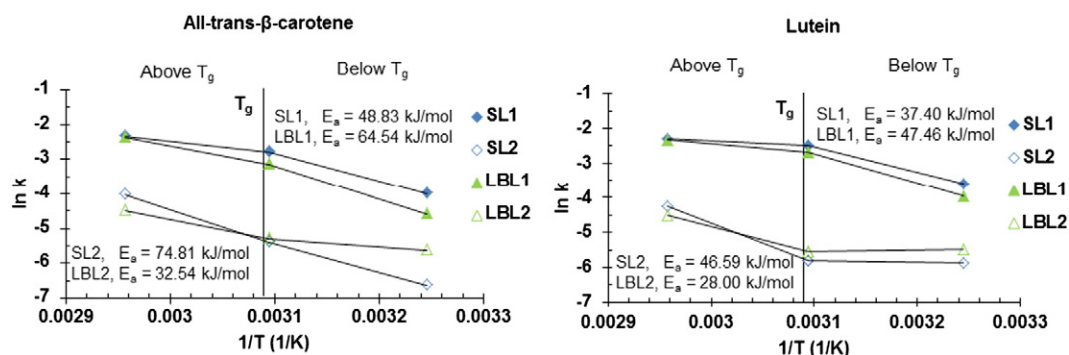


Fig. 7. Arrhenius plots of all-trans- $\beta$ -carotene and lutein in single layer (SL) and layer-by-layer (LBL) powders stored for 78 days at 35 °C, 50 °C, and 65 °C having a rapid initial first order loss kinetic (SL1 & LBL1) followed by a second slower first order loss kinetic (SL2 & LBL2) and showing the activation energies and glass transition temperature ( $T_g$ ).

Discontinuity between carbohydrate and protein used as wall materials occurred in this present study due to the presence of surface-active WPI. As a result, the carotenoids containing oil will be encapsulated within the glass former and non-glass former during spray drying. The lesser amount of carotenoids containing oil trapped and protected within the denser glass former increased the loss of carotenoids. Besides, the high amount of protein present in the continuous phase of the emulsion results in surface indentations of the powders obtained (Fäldt & Bergenstahl, 1994; Wang et al., 2013). The presence of WPI at particle surface increased the skin flexibility of the particles preventing skin rupturing during drying resulting in more wrinkles and surface roughness as observed in Fig. 2 and is commonly observed in spray dried milk and milk containing products (Wang & Langrish, 2010). This increases the effective total surface area of the powder particles allowing more surface area for heat transfer resulting in the higher loss of all-trans- $\beta$ -carotene and lutein. It was also noted that the loss of lutein was slightly more rapid than all-trans- $\beta$ -carotene based on the rate constant. The presence of two hydroxyl groups increased the polarity and hydrophilicity of lutein (Farombi & Britton, 1999; Updike & Schwartz, 2003). As a result, lutein will be more likely to assemble at the WPI-oil interface while all-trans- $\beta$ -carotene will be present within the bulk oil. It can be seen in Fig. 2 that lutein was present at the interfaces within a cut particle and appeared in large quantities on surfaces while all-trans- $\beta$ -carotene was dissolved within the encapsulated oil and was detectable only in small quantities on the SEM images. The location of lutein in oil particles allows lutein to be more susceptible to heat from the surroundings as well as trapped oxygen within the powder particles resulting in the faster degradation rate.

It can be observed that the degradation rate of all-trans- $\beta$ -carotene and lutein was faster in SL system than in LBL system in the initial rapid first order loss kinetic, and at higher storage temperature above the  $T_g$  in the second less rapid first order loss kinetic. The denser and thicker interfacial layer of the LBL particles, lower van der Waals attractive forces, and higher steric repulsions contributed to the higher stability of LBL systems than SL systems (Bouyer et al., 2011; Gu et al., 2005; Harnsilawat et al., 2006; Moreau et al., 2003). This can be attributed to the higher stability of LBL system towards heat from the surroundings due to the application of LBL layered interface. The LBL layered interface reduced heat transfer from the surrounding towards the encapsulated carotenoids reducing loss. On the other hand, the higher amount of surface oil on SL powders resulted in the higher loss of carotenoids in the SL system during the initial rapid first order loss. The significantly smaller particle size ( $d[4,3]$ ) of SL powders too results in higher carotenoid loss in SL system as the increased effective total surface area of the powders permits higher amount of heat transfer. The total retention of all-trans- $\beta$ -carotene and lutein in SL and LBL systems upon storage for 78 days (Fig. 8) also showed that the retention of carotenoids was significantly higher in LBL powders at higher storage temperature (65 °C). This indicated that the application of LBL layered interface was capable in providing the encapsulated carotenoid containing oil in LBL system with

significantly better protection against the loss of carotenoids at high storage temperature. The higher stability of LBL system towards environmental stresses such as heat, changes in pH, ionic strength, lipid oxidation, and freeze-thaw cycles too have been reported in several other studies (Iwata, Neves, Watanabe, Sato, & Ichikawa, 2014; Gharsallaoui et al., 2010; Güzey & McClements, 2006; Ogawa et al., 2003a; Ogawa, Decker, & McClements, 2003b). However, no significant difference was observed in the total retention of carotenoids between SL and LBL systems stored below and in the vicinity of the  $T_g$ .

#### 4. Conclusions

Spray drying of high total solid single layer (SL) and layer-by-layer (LBL) emulsions with a mixture of trehalose and WPI as wall materials produced high quality powders. The powders appeared similar visually as well as under light microscope observation. There was also no significant difference in the particle density, occluded air, interstitial air, bulk density and tapped bulk density between SL and LBL powders. The degradation of all-trans- $\beta$ -carotene and lutein increased with increasing storage temperatures and followed first order loss kinetics. The presence of two step loss kinetics of all-trans- $\beta$ -carotene and lutein showing a fast initial first order loss kinetic followed by a second less rapid first order loss kinetic was observed. There was reduced loss of carotenoids above the glass transition temperature ( $T_g$ ) based on the rate constants in the initial rapid first order loss kinetics. The loss of carotenoids in LBL system was more temperature dependent as LBL system has higher activation energies than SL system. Nonetheless there was a shift in the activation energies in the second first order loss kinetic as the loss of carotenoids in SL system became more temperature dependent than LBL system. Overall, the application of LBL interfacial structure on the oil particles reduced the loss of carotenoids in the initial rapid first order loss kinetic, and at higher storage temperature above the  $T_g$  in the second less rapid first order loss kinetic. The retention of carotenoids was also significantly higher in LBL powders at a higher storage temperature (65 °C) upon storage for 78 days indicating that the application of LBL interfacial structure provided the encapsulated carotenoid containing oil in LBL system with significantly better protection towards the high storage temperature. Minor changes occurred in the  $L^*$  (lightness) and  $a^*$  (redness) while bigger changes occurred in  $b^*$  (yellowness) values upon storage. No significant difference was observed in the water activity ( $a_w$ ) of both SL and LBL systems before and after storage at all storage temperatures. Data obtained from this current study is highly applicable in the food and pharmaceutical industries to increase the stability of oil soluble bioactives.

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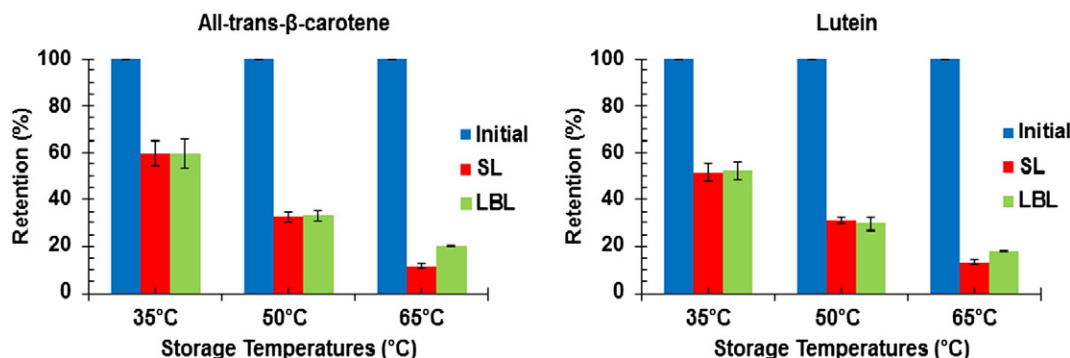


Fig. 8. Retention (%) of all-trans- $\beta$ -carotene and lutein in spray dried single layer (SL) and layer-by-layer (LBL) emulsions before and after storage at 35 °C, 50 °C, and 65 °C for 78 days.

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