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Contributed Paper

Characteristics and Flavor Retention of Structured Emulsion from Pomelo (*Citrus maxima*) Residue

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ABSTRACT

A growing awareness of consumer health has currently led to the increasing demand for healthy foods. To address this trend, food recipes have moved to products with lower fat content. According to our previous work, pomelo (*Citrus maxima*) fiber gel showed a promising property as a fat replacer. However, its retention of hydrophobic flavors like ethyl butanoate and ethyl hexanoate is still relatively low, compared to whipping cream which is composed of 35% fat. To overcome such a drawback, structured emulsions based on this fiber gel were designed and their characteristics, in particular flavor retention, were investigated in this study. The results revealed that the pomelo fiber is a rich source of dietary fiber comprising pectin and phenolic compounds that are able to claim an added health benefit. Moreover, the presence of pomelo fiber in the structured emulsions altered viscosity of the system from liquid-like to that of a gel with a storage modulus (G′) of 600 Pa, and an altered microstructure. Furthermore, the structured emulsions containing low of oil significantly decreased the volatility of the flavor compounds and affected their retention profiles, such that, the retention of hydrophobic flavors in the structured emulsions with 2.5% oil was found to be comparable to that of whipping cream. Such phenomena should thus be useful when formulating low-fat food products.

Keywords: flavor retention, structured emulsion, pomelo gel, fat replacer, microstructure, rheology

1. INTRODUCTION

Driven by consumer health concerns, reduced- or low-fat food products with physicochemical and sensory properties similar to those of their full-fat counterpart are highly desirable in the market. As such, some lipids in foodproducts may be replaced by reformulations with selected ingredients to maintain texture and rheological properties of the light products. Most of those ingredients do not provide any health benefits while dietary fiber based fat replacers provide special positive physiological benefits in disease prevention, particularly colon cancer and heart disease [1]. As a

consequence, a number of fat replacers based on fibers have been commercially launched [2] and some such as inulin [3], β-glucan from spent brewer's yeast [4], konjac glucomannan [5], oat dextrin [6] and orange by-product [7] have also been studied in various low-fat food models.

In addition to texture, the reduced- or low-fat foods should be designed to have similar sensory attributes (appearance, flavor, mouthfeel) and shelf life as conventional products, otherwise they will not be accepted by consumers. To serve such requirement, structural design principles has been recently used to formulate the healthy foods. For examples, oil-filled hydrogel particles obtained from biopolymer phase separation method [8], thermodynamic incompatibility [9] and complex coacervation [10] have shown the ability to mimic the desirable textural attributes normally provided by fat droplets. In addition, the applications of oil-in-water (O/W) emulsion-filled gels [11-12], water-in-oil-in-water (W/O/W) emulsions [13], and air-filled emulsions [14] have been reported for fat replacement in food products.

According to our previous work, pomelo (*Citrus maxima*) albedo was shown to be a promising starting material for pectin production [15]. Besides pectin, a solid residue containing pomelo fiber is usually generated as a waste during the pectin production, as shown in Figure 1. To approach a zero-waste process and to add more value to such waste, a fat replacer based on the pomelo fiber was successfully developed [16]. Nevertheless, our preliminary study showed that its retention of hydrophobic flavors is still relatively low when compared to high-fat food products like whipped cream. In this current study, a dietary fiber-based fat replacer was therefore developed through the structural design. O/W emulsions with oil contents of 0.5, 2.5, and 10% were embedded in the pomelo fiber gel matrix to form structured emulsions. Their properties, microstructures, and flavor-release characteristics were then investigated and compared with their emulsion and gel counterparts.

2. MATERIALS AND METHODS

The pomelo fiber was obtained as a by-product of pomelo pectin production according to the method of Methacanon *et al.* [15]. Sunflower oil (Angoon™) and whipping cream (Anchor™) comprising 35.50% fat (as labeled) were purchased from a local supermarket and used as received. Ethyl acetate (J.T. Baker), ethyl butanoate (Acros), and ethyl hexanoate (Acros) were used as model flavor compounds. Ethanol (HPLC grade, Merck) was used as solvent for the flavor mixture. All other chemicals used were of analytical grade.

2.1 Characterization of Pomelo Fiber

Soluble and insoluble dietary fiber contents in the pomelo fiber were determined by the enzyme-gravimetric method described by AOAC official method 993.19 and 991.42, respectively [17]. Water and oil holding capacities of the fiber were examined according to de Moraes Crizel *et al.* [7]. Total phenolic content present in the fiber was determined using the Folin-Ciocalteau assay with slight modification [7], as follows: dried sample (1.5 g) was homogenized with methanol (30 ml) using a high-speed dispenser (Ultra-Terrax® T25, IKA) for 2 min and subsequently centrifuged at 3000× g for 20 min at 4 °C. The extract (0.25 ml) was then diluted with deionized water (4 ml), followed by 10% v/v Folin-Ciocalteau reagent (Sigma, 125 µl). After 5 min, 7.5% w/v Na_2CO_3 (625 µl) was added. Then, the mixture was incubated for 2 h at room temperature before absorbance of the sample was recorded using a UV-visible

spectrophotometer (V-530, Jusco) at 725 nm. The total phenolic content was determined using gallic acid (GAE) as a standard and expressed as microgram of GAE per gram of dried sample. Functional groups of the pomelo fiber was revealed by the Nicolet 6700 FTIR spectrophotometer with a resolution of 4 cm^{-1} and 32 scans. The spectrum was recorded in the Attenuated Total Reflectance (ATR) mode in the range of 4000-400 cm^{-1}.

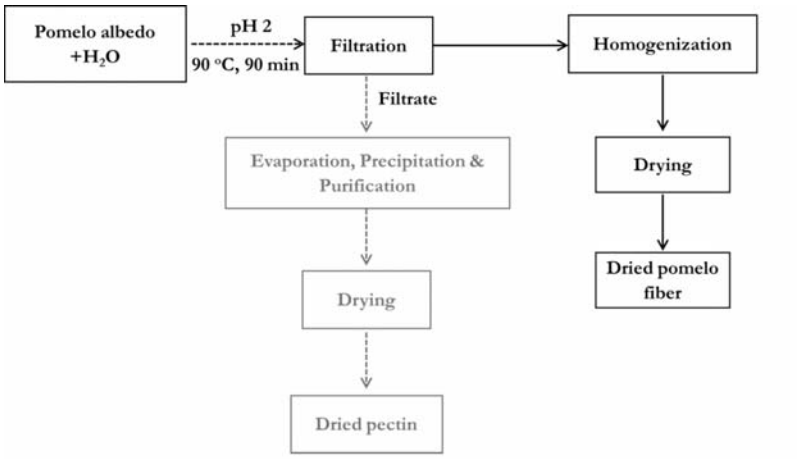

Figure 1. Pomelo fiber as a by-product from pomelo pectin production.

2.2 Preparation of O/W Emulsion

The primary oil-in-water (O/W) emulsion was prepared by dispersing 0.25-10% (w/w) of the oil phase (comprising sunflower oil and the three ethyl esters in ethanol) into the water phase along with Tween20 as a surfactant, using a high-speed disperser (T25 digital Ultra Turrax®: IKA, Germany) at 15,000 rpm for 30 s to form a coarse emulsion. Subsequently, the coarse emulsion was homogenized by being passed through a high-pressure homogenizer (APV1000, SPX, Denmark) at 600 bar for 2 cycles, to obtain the final O/W emulsion for further use. The final O/W emulsions (LE) consisted of 0.25-10% sunflower oil, 5% ethyl esters mixed solution (400 ppm of each ester in ethanol), and 2% Tween 20 (by wt.) in water.

2.3 Preparation of Structured Emulsion

Dried powder of the pomelo fiber was dispersed in water for at least 18 h to achieve complete swelling before being mixed with the prepared O/W emulsion (at twice the concentration listed in section 2.2) at a ratio of 50:50 (by wt.) for 2 h in a gas-tight bottle. The mixture was homogeneously blended using a blender (HR1613, Philips, China) for 2 min at room temperature to form the final structure. The final structured emulsions (SE) consisted of 5 % pomelo fiber, 0.25-10% sunflower oil, 5% ethyl esters mixed solution (400 ppm of each ester in ethanol), 2% Tween20 (by wt.) in water. 5% Pomelo fiber gel (PG) with 5% of the same 400ppm flavor mixture solution was also prepared by blending in the same manner described above. Whipping cream (WC) was used straight from the refrigerator and whipped at ambient temperature using a HR1613 Philips mixer equipped with a wire whip, operating at the speed of 2,000 rpm until stiff peaks appeared.

2.4 Rheological Analysis

Rheological measurements on the O/W emulsions (LE), their structured emulsions (SE), and the pomelo fiber gel (PG) were performed using a Gemini HR nano stress-controlled rheometer (Malvern Instruments, UK) with a serrated plate geometry (25 mm diameter and 1,000 mm gap size). Shear stress sweep tests at a constant frequency of 1 Hz were performed to determine the range of linear viscoelastic region (LVR) using shear stress between 0.1-100 Pa. Subsequently, dynamic frequency sweep tests were carried out between 0.1-100 Hz assuming a constant stress of 5 Pa (determined to be within LVR). Finally, steady shear experiments were performed over the shear rate range of 0.01-100 Hz. All tests were carried out at 25 °C and two replicates were performed for each test. The flow curves were fitted with the Herschel-Bulkley model, defined as

$$\tau = \tau_0 + K\dot{\gamma}^n$$

Where t is shear stress (Pa), t_0 is yield stress (Pa), $\dot{\gamma}$ is shear rate (s^{-1}), K is consistency coefficient (Pa s^{-n}), and n is flow behavior index.

2.5 Particle Size Distribution

Oil droplet size was determined using a MasterSizer 2000 (Malvern Instruments, UK). Samples were diluted in the instrument chamber with deionized water until the laser obscuration value was in the range of 20-25%. The following instrument parameters were set up: refractive index of the particles as 1.46, sample absorption as 0.1, and refractive index of the solvent (water) as 1.33. The average droplet size from three replicates was given in terms of the volume-based mean diameter (D[4, 3]).

2.6 Microstructure Analysis

Microstructures of the pomelo fiber gel (PG), the liquid emulsion (LE) and the structured emulsion (SE) were observed through an inverted fluorescence microscope (Olympus IX71, Japan) at 20× magnification. Nile red (1 mg/ml in ethanol, Sigma) used for staining oil was mixed with the samples (1 ml) and left for 1 h at room temperature. Subsequently, each sample was deposited onto a microscope slide and covered with a glass cover slip for inspection. The excitation wavelength used was in a range of 515-560 nm. Additionally, a confocal laser scanning microscope (Nikon eclipse Ti, Japan) was used to examine the microstructure of the structured emulsions at 100× magnification, where SE samples stained with Nile red were excited at 488 nm. All images were analyzed and processed using the NIS-elements Viewer software.

2.7 Retention of Flavor

For each sample, a series of the sample were transferred into 20 mL headspace vials at 4, 2, 1, and 0.5 g (4 vials for each amount) and capped immediately with silicone/PTFE septum-lined caps (National Scientific Co., USA). The amount of time required to establish an equilibrium of flavor molecules between the sample and the air phases in the headspace vials at 50 °C had previously been determined to be 20 min in preliminary experiments. Furthermore, the partition coefficients in water matrix of single ester solutions and of three-ester mixture solution were determined, and the difference in their values were found to be insignificant ($p > 0.05$), which indicated that there were no interactions between the individual esters at the concentrations used. The samples in headspace vials were thus incubated at 50 °C for 20 min in an autosampler,

immediately after which the headspace air sample (1 ml) was withdrawn into a preheated (75 °C) 2.5 mL gas-tight syringe (CTC Analytics, Switzerland) and injected into a gas chromatograph (GC-MS-QP2010, Shimadzu Inc., Japan) in split mode (split ratio 4:1). The gas chromatograph was equipped with an Rxi-1MS capillary column (60 m, 0.32 mm i.d., 1 µm film thickness) and coupled with an MS detector. The injector and detector temperatures were 210 and 230 °C, respectively. The helium carrier gas column flow rate was 1.2 mL/min. The temperature program used was 35 °C (10 min) to 200 °C (5 min) at 5 °C/min heating rate. The partition coefficients of the three ethyl esters in each sample matrix (K_{sample}) and in water matrix (K_w) were determined using the phase ratio variation (PRV) method [18] with the goodness of fit ($r^2 > 0.9950$). The experiment for each sample matrix was carried out in quadruplicates. The percentage of flavor retention (R) of each ester in each matrix, with water as the reference matrix, was then calculated according to the following equation [18].

$$R\ (\%) = [\ 1 - \frac{K_{sample}}{K_w}\ ] \times 100$$

3. RESULTS AND DISCUSSION

3.1 Characteristics of Pomelo Fiber

The amounts of insoluble (IDF) and soluble (SDF) dietary fibers in the pomelo fiber, as determined by the enzyme-gravimetric method, were 44.79% and 23.03% based on dry matter, respectively. As a consequence, total dietary fiber (TDF) was approximately 68%, suggesting that the pomelo fiber is a rich source of dietary fiber [19]. Although IDF assigned as cellulose and hemicelluloses was the largest fraction, a relatively high amount of SDF was also found. This indicated that some pectin were still left in the pomelo fiber after pectin extraction process. The presence of pectin in pomelo fiber was also supported by the FTIR spectrum in Figure 2. The absorption bands at 1613 cm^{-1} and 1736 cm^{-1} assigned as carboxylic acid (-COOH) and its esterified group ($-COCH_3$), respectively, are characteristic of pectin. In addition, those at 3285 $cm^{-1}$and 2895 cm^{-1} belonging to hydroxyl (-OH) and methyl groups ($-CH_2$) are characteristic bands of polysaccharides [15]. Besides pectin, total phenolic acids were detected as 336.26 ± 6.27 µg/g pomelo fiber. It has been reported that phenolic compounds, particularly those derived from plant, are more effective antioxidants *in vitro* than vitamins E or C, and might contribute significantly to the protective effects *in vivo* [20]. Moreover, the phenolic compounds were mainly found in citrus fruit peels rather than their fruits [21]. Since water-holding and oil-retention capacities are functional properties of dietary fibers, such properties of the pomelo fiber were determined as 8.46 ± 0.34 g water/g dry sample and 1.86 0.06 g oil/g dry sample, respectively. With its high hydration capacity, the pomelo fiber could be used as a functional food ingredient to reduce calories and syneresis as well as to modify viscosity and texture of the final food products, while the oil retention capacity is beneficial for flavor retention [22]. Furthermore, it was associated with the phenolic compounds, which may provide additional health-promoting effects.

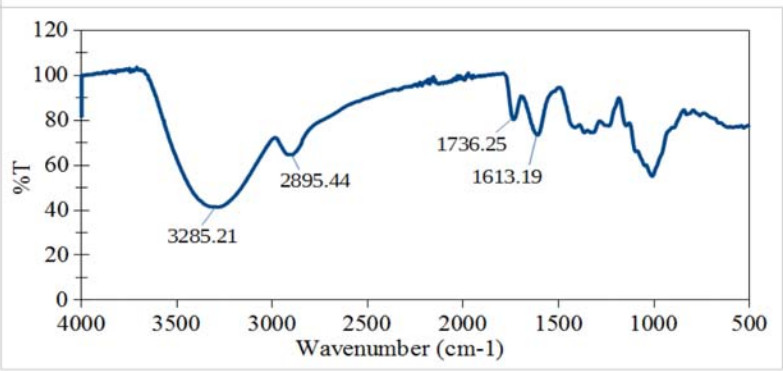

Figure 2. FTIR spectrum of pomelo fiber.

3.2 Particle Size and Microstructure

Since this work was aimed towards low-fat food products, a relatively low maximum oil content of 10% in the emulsions was selected for investigation. The particle size distributions of the pure pomelo fiber gel (PG), the O/W emulsion (LE) containing 10% sunflower oil, and the corresponding structured emulsion (SE) are shown in Figure 3. Results showed that distribution of oil droplets in LE sample was bimodal, with particle size ($D_{4,3}$, the volume-based mean diameter) ranging from 0.15 to 2.72 μm. On the other hand, the pure PG showed a mono-modal distribution with significantly larger average particle size ($D_{4,3}$) of 135.76 ± 0.97 μm, which is relatively similar to the average particle size observed in the SE sample (118.79 ± 0.28 μm). This indicates that the presence of oil droplets in the SE sample did not contribute to a major change in the particle size compared to that of PG, and implies that the emulsion droplets were well embedded into the pomelo fiber gel matrix. However, this result is somewhat inconsistent with those of previous works, which showed bimodal distributions in their emulsion-filled gel systems [8, 23].This may be due to the lower viscosity of their emulsion systems, leading to the observed bimodal size distribution due to fat droplet aggregation.

Microstructure analysis of LE (O/W emulsion with 10% sunflower oil), PG (pomelo fiber gel), and SE (structured emulsion) was performed using fluorescence microscopy. The oil phase was stained with Nile Red; hence, the oil droplets in LE appeared as yellow bright spherical droplets as shown in Figure 4a. The result clearly shows that oil droplets in the emulsion were separated and evenly distributed across the image, indicating no droplet flocculation or coalescence. Furthermore, the sizes of the LE droplets observed under the microscope (approximately 1 μm) correspond well with the droplet size measured using the MasterSizer 2000 mentioned earlier. In the case of the pure pomelo fiber gel (PG), coarse strands characteristic of fibers were clearly visualized (Figure 4b). Apart from the fiber strands, the spherical droplets with diameters larger than approximately 10 μm are likely air-trapped hydrogel bubbles formed by the soluble dietary fiber component of our pomelo gel system. To clearly observe the oil phase embedded inside and distributed throughout the fiber gel in the structure emulsion sample (SE), a confocal laser scanning microscopy (CLSM) was used. The CLSM images (Figure 4c and 4d) showed a coarsened microstructure

associated with PG and luminescent oil droplets (approximately 1 µm) embedded inside and distributed throughout the fiber gel.

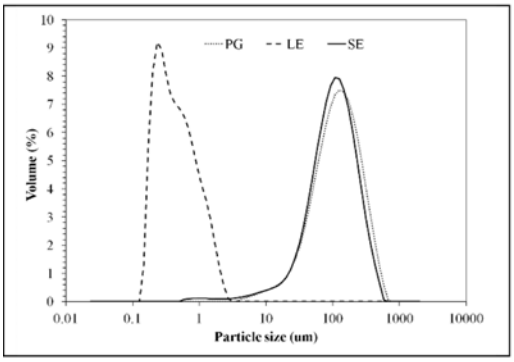

Figure 3. Particle size distribution observed in PG (pomelo fiber gel without emulsion, ····), LE (O/W emulsion with 10% oil, ——), and their associated SE (structured emulsion, ——).

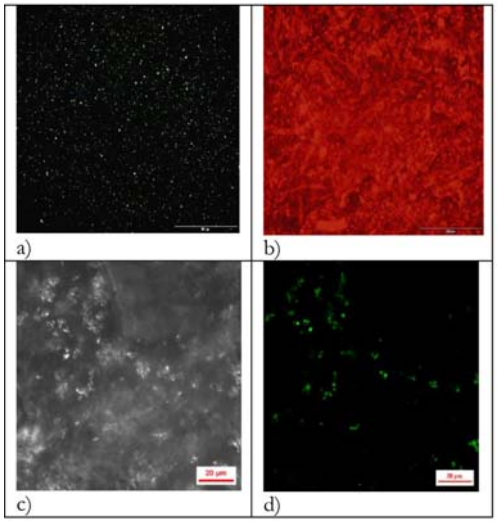

Figure 4. Fluorescence micrographs of (a) O/W emulsion with 0.5% sunflower oil, (b) pomelo fiber gel, scale bars = 200 µm and CLSM images of structured emulsion (SE) with 10% sunflower oil: (c) bright field, and (d) Nile red staining, Scale bars = 20 µm.

3.3 Rheology

Rheological properties of the pomelo fiber gel (PG), O/W emulsions (LE) with various oil contents (0.25-10%), including their corresponding structured emulsions (SE) were measured and shown in Figure 5. The LE samples exhibited the Newtonian flow behavior (Figure 5a) while PG and SE showed shear-thinning over the shear rate range used, with a power law index (n) of approximately 0.13 ± 0.02 (Figure 5b). Viscoelastic properties of the O/W emulsions could not be measured due to fluidity of the samples, but the PG and SE samples had structures and exhibited a gel-like behavior with storage moduli (G′) higher than loss moduli (G″), as shown in Figure 5c. Moreover, their value were almost independent of frequency over the entire frequency range (Figure 5d). It is also worth noting that the oil contents (0.25-10%) likely had no significant effect on the rheological properties of SE samples, as demonstrated by the similar consistency indices that were obtained (K = 54.81 ± 5.47). Due to the relatively low oil contents (≥ 10%) used in this study, the obtained results are slightly inconsistent with several other works, which reported that the rheological properties and breakdown behavior of gels filled with emulsion droplets depended on gel matrix type, oil content, droplet size, and interaction between oil droplets and gel matrix [24]. Nonetheless, the higher the oil content, the lower the rheological properties, particularly viscosity, tended to be.

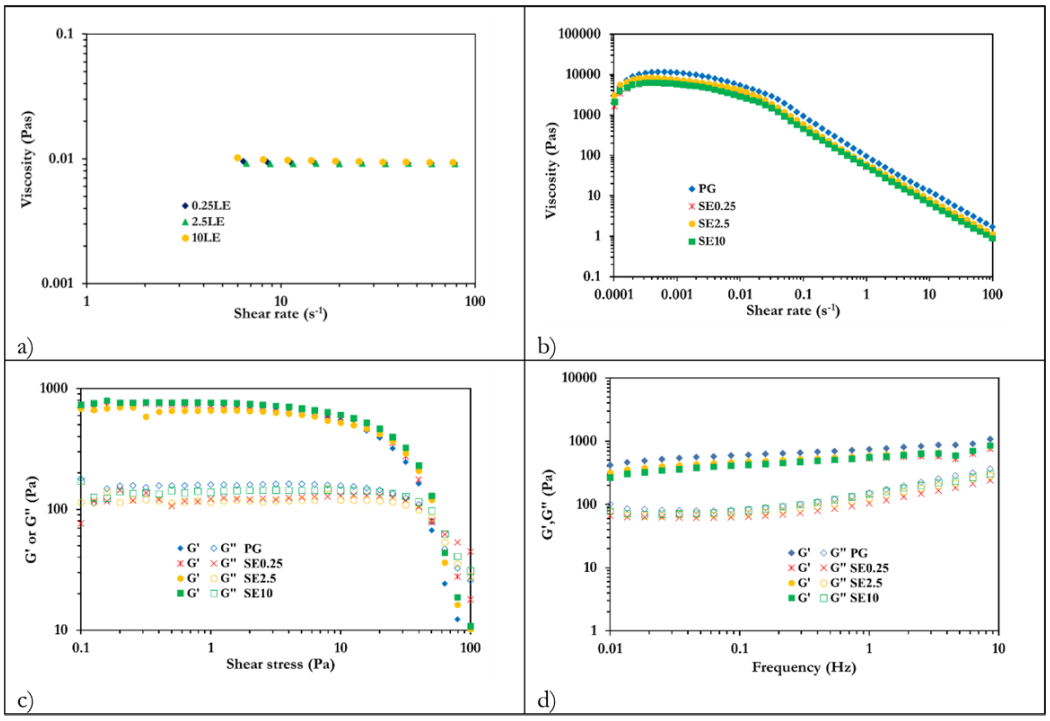

Figure 5. Flow behavior of O/W emulsions (LE) with oil various contents (0.25, 2.5, and 10%) (a), their corresponding structured emulsions (SE) and pomelo fiber gel (PG) (b). Storage (G′: filled symbols) and loss (G″, open symbols) moduli as a function of stress (c) and as a function of frequency (d) for PG and SE samples.

3.4 Flavor Retention

Flavor release from food is mainly controlled by two factors: the volatility of flavor compounds (a thermodynamic factor) and the resistance to mass transfer from food matrix to air phase (a kinetic factor). The thermodynamic factor determines the retention or partition of flavor in the matrix at equilibrium, and the kinetic factor mainly affects the release rate of flavor from foods. These factors are largely influenced by the interactions between flavor compounds and food ingredients and by environmental conditions under which flavor release is triggered [25]. In other words, there are many factors affecting retention and release of flavor in foods, such as viscosity [26], microstructure and composition of food matrix, and physicochemical properties of flavor compounds [27]. Three linear esters, namely ethyl acetate, ethyl butanoate, and ethyl hexanoate, were selected as the model flavor compounds for this study.

As shown in Table 1, they are considerably different in their physicochemical properties. With increasing carbon chain length, log P (the octanol/water partition coefficient, representing the affinity for oily phase) increases, whereas solubility in water (which corresponds to hydrophilic character of the molecule) decreases. The air/water partition coefficients (K_w) of the three ethyl esters, which represent the volatility of these molecules in water, were firstly determined. The order of the resulting K_w values was: ethyl acetate < ethyl butanoate < ethyl hexanoate, which is in good agreement with the increasing carbon length in the alcohol moiety of the

esters. This behavior can be explained by their solubility in water: with increasing hydrophobic alkyl length in the esters (reduction in the polar character), the water solubility consequently becomes lower, resulting in higher relative volatility. Secondly, these flavor compounds were then incorporated into the O/W emulsions (LE) consisting of different oil contents (0.25, 2.50, and 10%w/w) and their partition coefficients were subsequently evaluated. The results (Figure 6) clearly showed that the presence of the O/W emulsions affected the partition coefficients of the flavor molecules, particularly ethyl hexanoate: the higher the oil content, the lower the partition coefficient value tended to be. Moreover, among the three studied flavor compounds, ethyl hexanoate appeared to be the most volatile compound in water but the least volatile one in the emulsions. In other words, the presence of oil in the O/W emulsions (LE) offered the biggest flavor retention enhancement for ethyl hexanoate, in contrast to ethyl acetate which showed the lowest retention improvement when compared to water matrix. It is also worth noting that only small amount of oil (0.25%) in the emulsion resulted in markedly decrease the partition coefficient of ethyl hexanoate, compared with that in water. This indicated that the oil content in the emulsions decreased the concentration of hydrophobic volatiles in the headspace and therefore increased the flavor retention. On the other hand, the presence of oil in emulsions imparted less retention enhancement for ethyl acetate, possibly due to the somewhat higher polarity of ethyl acetate.

Based on the partition coefficient of each flavor compound, the corresponding percentage of retention (R) in the studied matrices (relative to water) was calculated and shown in Figure 7. The retention tended to increase with the carbon length of the esters. This is in agreement with the "Thijssen selective diffusion" theory, which states that while moisture continues to evaporate, the evaporation of volatiles with larger molecules becomes more limited due to diffusion [29]. By comparing the R values between the emulsion (LE) and the structured emulsion (SE), it can be deduced that the viscosity of system also had a pronounced effect on the retention of flavors in particular ethyl acetate: diffusion of flavor molecules was reduced as the solution viscosity increased (data not shown). To clearly illustrate this point, the retention of ethyl acetate in the O/W emulsion (LE) containing 0.25% oil was approximately 31%, while the value went up to approximately 69% in the corresponding structured emulsion (SE). This illustrates that volatility of flavor molecule is likely obstructed by the barrier formed by high-viscosity matrices [30] and specific binding interaction with thickeners [29].

Table 1. Physicochemical properties of ethyl esters used in this work.

	Ethyl acetate	Ethyl butanoate	Ethyl hexanoate
Structural formula	 $C_4H_8O_2$	 $C_6H_{12}O_2$	 $C_8H_{16}O_2$
MW (g/mol)	88.11	116.16	144.21
Log P [28]	0.7	1.8	2.8
Water solubility (g/L) [28]	67.1	5.3	0.5

MW = molecular weight; Water solubility at 37 °C.

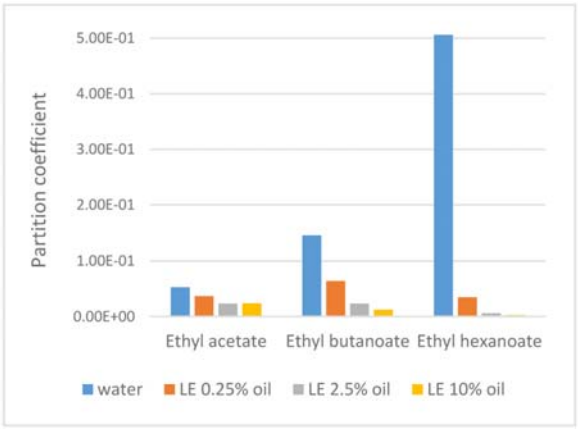

Figure 6. Partition coefficients of ethyl acetate, ethyl butanoate, and ethyl hexanoate in water and in O/W emulsions (LE) with varying oil contents (0.25-10% w/w).

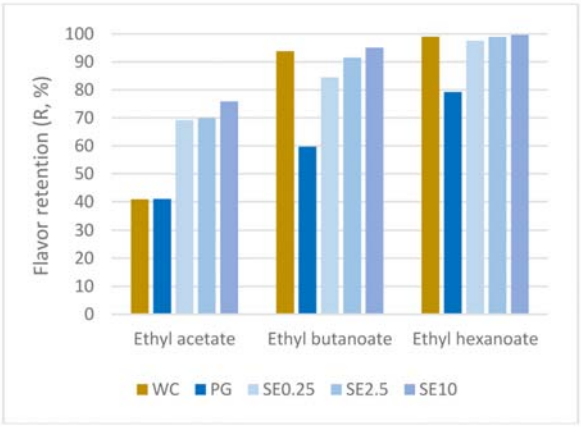

Figure 7. Retention of flavors in whipped cream (WC), pomelo fiber gel (PG), structured emulsions (SE) with varying oil contents (0.25-10% w/w).

Besides the viscosity, oil content and flavor-lipid interaction also influenced the flavor retention. The comparison between pure pomelo fiber gel (PG) and the structured emulsion (SE) series strongly exhibits this observation: with similar viscosity, flavor retention of samples can be significantly enhanced by the presence of oil. This is because, in samples containing oil, hydrophobic aroma compounds are bound to fat molecules by weak, reversible Van der Waals and hydrophobic interactions. As a result, their vapour pressures reduce, leading to decrease a flavor concentration in headspace. Hence, it should be noted that changing fat contents in food products, even small quantities, may lead to imbalanced flavors and affect food acceptability.

Of interest is flavor retention of the structured emulsions compared with that of whipped cream (WC) containing high fat content (ca. 35%). As shown in Figure 7, more than 90% of ethyl butanoate and ethyl hexanoate, which are relatively non-polar, were retained the whipped cream, while ethyl acetate was retained by only 40%. In contrast, the structured emulsions retain ethyl acetate better than the whipped cream, possibly due to the fact that pomelo fiber gel possesses highly polar functional groups, such as

hydroxyl and carboxyl groups normally present in pectin. For retention of ethyl butanoate and ethyl hexanoate, the emulsions structured by pomelo fiber gel containing only 2.5% oil was comparable to that of whipped cream consisting of approximately 35% fat.

4. CONCLUSIONS

The O/W emulsions structured with pomelo fiber gel (SE) exhibited promising textural and flavor properties towards applications in reduced/low-fat food products. It is worth noting that characteristic of flavor molecules, oil content and matrix viscosity affects the flavor retention. Compared with pure pomelo fiber gel (PG), a relatively small amount of oil (0.25%) present in the SE samples helped enhance retention of ethyl acetate from 41 to 69%, ethyl butyrate from 60 to 84%, and ethyl hexanoate from 79 to 98%. Furthermore, the results showed that increasing the viscosity of the system enhanced the retention of the flavor compounds. Due to the relatively high polarity of PG, SE samples containing 0.25-10% oil retained ethyl acetate better than whipped cream consisting of 35% fat, while SE with 2.5-10% oil retained ethyl butanoate and ethyl hexanoate at comparable capacities to the whipped cream.

ACKNOWLEDGMENT

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