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Rheological behaviour of emulsions of avocado and watermelon oils during storage

T.V. Logaraj [a,*], Suvendu Bhattacharya [b], K. Udaya Sankar [b], G. Venkateswaran [a]

[a] Food Microbiology Department, Central Food Technological Research Institute, Mysore 570020, India
[b] Food Engineering Department, Central Food Technological Research Institute, Mysore 570020, India

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Abstract

Rheological properties of emulsions made out of avocado pulp and watermelon seed oils with whey protein concentrate were determined during different storage periods. The oils, as well as the emulsions behaved like non-Newtonian liquids, having shear-thinning characteristics. Both oils showed moderate shear-thinning characteristics as the flow behaviour indices were between 0.86 and 0.88. The shear-rate/shear-stress data could be adequately fitted ($r = 0.997$–0.999) to a common rheological equation, e.g. the power-law model. Avocado pulp oil was markedly more viscous than was watermelon seed oil which was also evident from the higher apparent viscosity and consistency index values.

The rheological parameters during storage did not significantly change flow parameters, e.g. flow behaviour and consistency indices and apparent viscosity. The values of different droplet sizes and their distribution patterns, as evident from phase contrast microscopy, were considered almost unimodal (i.e., between 2 and 10 μm). Such a narrow range of variations in the particle diameters may not markedly influence particle behaviour. Cream and serum separation of emulsions were not noticed during storage, indicating that the stability of these two emulsions was not affected during storage for up to 2 months at room temperature.

Keywords: Rheology; Emulsions; Avocado and watermelon; Apparent viscosity; Flow behaviour index

1. Introduction

The importance of unsaturated fatty acids in foods is well recognized. The classes of unsaturated fatty acids include MUFAs (monounsaturated fatty acids), e.g. oleic acid (18:1) and PUFAs (polyunsaturated fatty acids) of the ω-6 and ω-3 families. The major dietary ω-6 PUFAs include linoleic (18:2), gamma linolenic (18:3), and arachidonic (20:4) acids, whereas major ω-3 PUFAs include alpha linolenic (18:3), eicosa pentaenoic (20:5), docosa pentaenoic (22:5) and docosa hexaenoic (22:6) acids (Garg, Wood, Singh, & Moughan, 2006).

Oleic acid (OA) is a MUFA (monounsaturated fatty acid) present as a major constituent in avocado oil. Recent researches indicate that MUFA reduces the levels of an oncogene called Her-2/neu (also known as erb-B-2) and is effective in controlling breast cancer cells. High levels of Her-2/neu occur in more than one-fifth of breast cancer patients and are associated with highly aggressive tumors that have a poor prognosis. Further, the presence of oleic acid boosts the effectiveness of trastuzumab (herceptin) and can help to prolong the lives of many such patients (Menendez, Vellon, Colomer, & Lupu, 2005). Linoleic acid is also a C-18 poly unsaturated fatty acid with two double bonds. Watermelon seed oil also contains this fatty acid content and is being used as a cooking oil, as well as a food additive, in western Africa and middle east countries.

Avocado oil is suitable for preventing the human body from accumulating the undesirable low-density lipoprotein (LDL) cholesterol and promotes healthy high-density lipoprotein (HDL) cholesterol accumulation, which is beneficial to the heart. Studies also prove that the presence of β-sitosterol in avocado oil helps in relieving the symptoms

* Corresponding author.
E-mail address: vlogaraaj@yahoo.co.in (T.V. Logaraj).

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of prostate enlargement amongst men, besides lowering the cholesterol build-up. It also contains other health-sustaining compounds, e.g. lutein and other colour pigments, which are implicated in the eye disorders of older people (Chan, 2005).

Equal amounts of long chain ω-3 and ω-6 unsaturated fatty acids were consumed by earlier human populations but newer agricultural practices have led to increased consumption of processed fats and oils, leading to a change in the ω-6 to ω-3 fatty acid ratio, which is now around 7:1–25:1. The consumption of long chain ω-3 fatty acids is decreasing the ratio of ω-6 to ω-3 and hence is effective in treating coronary heart diseases, type-2 diabetes, hypertension, immune response disorders and mental illness (Djordjevic et al., 2005). The required increase in PUFA intake can be achieved by consumption of PUFA-rich supplements. However, a few problems are encountered in the production, transportation and storage of these fortified foods as PUFAs are extremely susceptible to oxidative deterioration.

Techniques for controlling the oxidation of PUFAs in bulk oils include temperature control, oxygen-free atmosphere and addition of a lipid-soluble antioxidant. These fatty acids in oils also need to be protected when they are added to processed foods. Oil-in-water (o/w) emulsions are easy to disperse into water-based foods, e.g. beverages, dairy products, salad dressings and muscle foods, than into bulk oils that could physically separate from the aqueous phase upon storage. Emulsions also maintain similar physical structures when incorporated in water-based foods.

Whey protein isolate/concentrate (WPI/WPC) is a common food additive, often used to produce low viscosity oil-in-water emulsions at oil concentrations ranging from 5% to 30%, having excellent physical stability against thermal processing (Djordjevic, Kim, Mc Clements, & Decker (2004). These emulsion-based delivery systems need to be kinetically stable under a range of conditions which they might experience in food preparations, e.g. sterilization, pasteurization, cooking heat, pressure and during storage (Euston, Finnigan, & Hirst, 2000).

Avocado oil contains oleic acid (54–76%) and linoleic acid (11–15.6%) while watermelon seed oil contains linoleic acid (59.6%) and oleic acid (18.1%) as major components (El-Adawy & Taha, 2001; Vekiari, Papadopoulou, Lionakis, & Krystallis, 2004). The super critical CO_2 extraction (SCFE) of these oils provides the maximum composites of these unsaturated fatty acids without any solvent contamination. Owing to referred benefits, these two oils are used in the present study for the purpose of developing emulsions to be used as effective nutraceutical delivery systems.

The present study is aimed at developing WPC stabilized oil-in-water emulsions as effective ingredients for delivery systems, and to incorporate both MUFAs and PUFAs as nutraceuticals in functional foods. Hence, it would be advantageous to produce emulsions with high oil contents to reduce the cost of transportation, maximize concentration of bioactive lipids and with low viscous behaviour to facilitate ease of application. The developed product should also be stable against oxidation and possess desirable sensory quality attributes with a good shelf life. This may pave the way for developing physically and oxidatively stable ω-3 and ω-6 fatty acid delivery systems as emulsions, which only need to be added to foods in small quantities as fortifiers or as direct dosages having nutraceutical and therapeutic values.

The major use of an emulsion depends upon its rheological characteristics. It is thus important to understand the relationship between rheology and sensory perception during the process of mastication. The quality attributes may be described in terms of creaminess and stickiness, which could be linked to pourability and spreadability of emulsions. These in turn are linked to rheology. An understanding of the rheological behaviour of food materials, such as emulsions, is required for the purpose of product development, process selection and standardization, and to know the stability of the samples during storage. This is true for both food and drug preparations. An examination of the suitability of rheological models is also required for characterization of materials and for scale-up purposes. It may be mentioned here that the rheological behaviour of avocado and watermelon oils, and emulsions made out of them have not yet been reported; the importance of such studies lies in formulating the nutraceutically valued emulsions using these oils.

The concentration of protein in the o/w emulsion is critical for stabilizing the emulsion, and hence, affects the rheological status. Emulsified fat contributes to creaminess of the system in addition to viscosity (Richardson & Booth, 1993). The size and number of oil particles, and their distribution pattern also impart a significant effect on rheological characteristics; further, the state of aggregation of the droplets possibly has an influence (Depree & Savage, 2001). The size of oil droplets and the total interfacial area of the emulsions made under identical emulsifying conditions are not the same for different kinds of proteins (Walstra & de Roos, 1993). This difference is attributed to viscoelastic properties of the protein films formed at the o/w interface (Dickinson, 1999) and to the amount of protein adsorbed to the interface (Tcholakova, Denkov, Sidzhakova, Ivanov, & Campbell, 2003). Conventionally, the viscosity-related properties of liquid and semi-solid foods are assessed by measuring their resistance to flow. The rate of flow over the surface of mechanoreceptors and the amount of force required to manipulate the fluid in the mouth are also critical in this regard (Christensen & Casper, 1987).

In the present study, the system conditions have been optimized to avoid droplet aggregation or coalescence and to explore the general rheological behaviour, aiming for stability and quality of the product. Thus, a fine and uniform emulsion having a rheological stability is being attempted, that can be stored over a reasonable period of time for use while retaining the quality. This principle

has been employed for developing emulsions using avocado or watermelon oils.

The objectives of the present studies are to determine, (a) the rheological properties of emulsions made out of avocado pulp oil and watermelon seed oil, along with whey protein concentrate, (b) the stability of these emulsions during storage and (c) suitability of the model to predict their rheological behaviour.

2. Materials and methods

2.1. Materials

Whey protein concentrate (WPC), obtained from Mahaan Foods, New Delhi, India having about 93% protein, 5% moisture and 2% sulfated ash (dry weight basis), was used for the present study. Avocado fruits (*Persea americana* Mill.) and watermelon (*Citrullus lanatus Thunb. syn. Colosynthis citrullus* Linn.) seeds were procured from the Horticulture Department of Mysore, Karnataka, India.

2.2. Freeze-drying of pulp and seed material

Dry watermelon seeds were procured and stored at −20 °C in a deep freezer for one day, prior to grinding employing a laboratory grinder (IKA mini mill, Germany). For avocado fruits, containing about 80% water and 20% pulp (w/w), the pulp was converted into a puree by using a wet grinder, followed by freeze-drying by spreading in trays (45 cm × 90 cm) with a thickness of 2–3 mm. The puree was frozen at −30 °C and was freeze-dried (Model # Lyodrier LT5B, Lyophilization System, USA). The drying was conducted at 200–300 μm of vacuum with a drying temperature programmed from −20 to 25 °C. The total time taken for drying the material was 12 h.

2.3. Extraction of oil

Super critical carbon dioxide extraction of oils of both freeze-dried avocado puree and watermelon seeds was performed. The extraction was carried out by using a super critical CO_2 extractor (Model # EX – 4.2, Nova Swiss, Switzerland). The oils were extracted by using 100 g each of both avocado and watermelon materials, separately, below a temperature of 50 °C, and a pressure of 240 bars was maintained. The extraction was completed over a period of 12 h and about 55% of oils were recovered from both the samples (dry basis). This was devoid of any solvent impurity or intervention, and was used for preparing nutraceutical emulsions for further testing.

2.4. Emulsion preparation

Oil-in-water emulsions were made by following the method of Djordjevic et al. (2004). The SCF CO_2 extracted avocado pulp oil or watermelon seed oil was mixed with an aqueous phase having 10 mM sodium citrate buffer in the ratio 1:3, followed by adjustment of pH to 3.0, and WPC was added to obtain a final protein-to-oil ratio of 1:10 (i.e. 2.5 wt% protein for 25 wt% of oil). WPC was added, with the buffer, by stirring at 4 °C for 12 h to obtain an uniform dispersion. Prior to homogenization, the aqueous phase was stirred for 1 h at room temperature (25 °C) and was readjusted to a pH of 3.0 using 1.0 M HCl solution.

A high speed homogenizer of cup and cone system (Model # RQ –127 A/D, Remi, India), provided with a speed regulator, was used. The homogenizer had a toothed stator and rotor that were separated by a small clearance to offer a wide range of fine globules/particle sizes. A coarse emulsion premix was obtained, using this apparatus, by homogenizing oil and aqueous phase at a moderate speed for 2 min at room temperature (25–27 °C). The coarse emulsion was passed through a high speed range of about 5000 rpm for a period of 20 min. Immediately after homogenization, the formed emulsions were cooled by placing the samples in an ice bath.

The emulsions were heated in a water bath up to 75 °C, followed by a holding for 30 min to avoid microbial contamination. Antioxidant, in the form of mixed tocopherol isomers at a level of 200 ppm, was added prior to homogenization. Once the emulsions were formed, 10 ml of each sample were transferred to glass tubes with lids. The internal diameter of the tubes was 15 mm and a height of 125 mm, and the samples were stored at room temperature prior to further analyses.

2.5. Phase contrast microscopy

The emulsion samples were kept as a small drop over a glass slide with a cover slip, and were viewed at a magnification of 40×, employing a phase contrast microscope (Model # BX40/F4, Olympus Optical Company, Japan). The respective particle sizes and their distribution patterns were calculated for a population of 20 particles at a constant range of magnification. This particle characterization was within the limits of phase contrast microscopy and the size recognition started at about 2 μm only.

2.6. Rheometer and rheological measurements

A controlled stress (CS) rheometer (Model # RT10, Haake GmbH, Karlsruhe, Germany) with a coaxial cylinder attachment, having a ratio of 0.922 between the external diameter of the rotating bob and the internal diameter of stationary cylinder, was employed to determine the rheological behaviour of emulsions. The gap between the cup and rotor was 850 μm. A constant temperature of 20 ± 0.1 °C was maintained during the measurement using a circulatory water bath. The controlled rate (CR) measurement technique was employed by progressively increasing the shear-rate up to 500 s^{-1} to obtain 50 shear-rate/

shear-stress data points. All rheological measurements were conducted on triplicate samples.

2.7. Analysis of data and statistics

The shear-stress and shear-rate data were fitted to the power-law (Ostwald-de-Waale model) by Eq. (1), as shear-thinning was observed but not the existence of yield stress. The flow behaviour index and consistency index were estimated by employing non-linear analysis of shear-stress/shear-rate data, using the software supplied by the equipment manufacturer.

$$\sigma = k(\dot{\gamma})^n \quad (1)$$

Here, σ is shear stress (Pa), $\dot{\gamma}$ is shear-rate (s^{-1}), k consistency index (Pa s^n) and n is flow behaviour index (dimensionless). The apparent viscosity of the samples was obtained as the ratio of the shear-stress and shear-rate when the latter was taken to be 50 s^{-1}. The suitability of the rheological model, relating shear-rate and shear-stress values, was judged by determining the correlation coefficient (r) and chi-square values.

3. Results

The oils, as well as the emulsions, behaved like non-Newtonian liquids, having shear-thinning characteristics as shown by the sample rheograms (Figs. 1 and 2). Both oils had moderate shear-thinning characteristics as the flow behaviour indices were between 0.86 and 0.88. The shear-rate/shear-stress data could be adequately fitted (r = 0.997–0.999) to the common rheological equation, i.e. the Ostwald-de-Waale or power-law model. Interestingly, both possessed similar flow behaviour index values. Avocado pulp oil was markedly more viscous than was watermelon oil which is evident from its higher apparent viscosity and consistency index values. In addition, the apparent viscosity values showed good repeatability with standard deviation values hardly crossing 3 mPa s.

The emulsions containing whey protein concentrate (WPC) in avocado pulp or watermelon seed oils also showed shear-thinning behaviour, as indicated by the flow behaviour index values (0.570–0.619 and 0.534–0.633, respectively) that are lower than those of their corresponding oils (Tables 1, 2), meaning that the addition of buffer salts and WPC, in addition to the formation of an emulsion, makes the samples able to develop non-Newtonian characteristics (see Figs. 1 and 2).

Figs. 3 and 4 show the phase contrast photomicrographs of avocado and watermelon emulsions, respectively, kept for different storage periods. Avocado oil-based emulsions possessed small particles of diameter less than 5 μm (Table 3) prior to storage, and up to 30 days of storage, though the particle size increased during this storage period. At later periods, there was an occurrence of about 30% of

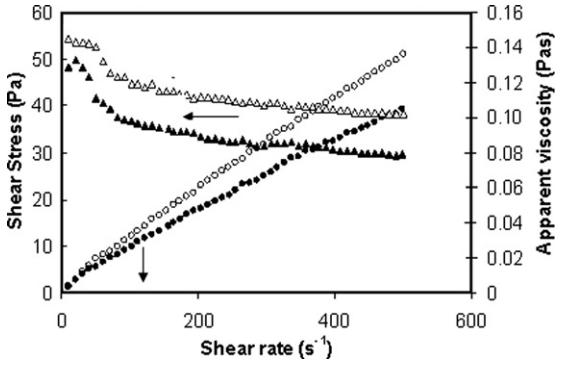

Fig. 1. Rheograms for watermelon seed oil and avocado pulp oil. Hollow symbols: avocado pulp oil; closed symbols: watermelon seed oil.

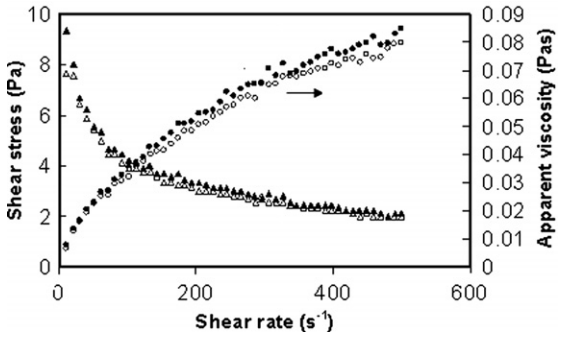

Fig. 2. Sample rheograms for emulsions of avocado pulp oil and watermelon seed oil with whey protein concentrate at the beginning of storage period. Hollow symbols: avocado pulp oil; closed symbols: watermelon seed oil.

Table 1
Rheology of oils used for the study

Type of oil	Apparent viscosity, η (Pa s)	Flow behaviour index, n (–)	Consistency index, k (Pa s^n)
Avocado	0.1647 ± 0.027	0.8809 ± 0.006	0.2595 ± 0.053
Watermelon	0.1125 ± 0.001	0.8599 ± 0.012	0.1906 ± 0.011

Table 2
Rheological status during storage of emulsions made using avocado pulp oil and watermelon seed oil

Storage period (days)	Apparent viscosity, η (Pa s)	Flow behaviour index, n (–)	Consistency index, k (Pa s^n)
Avocado pulp oil			
0	0.0504 ± 0.003	0.5934 ± 0.013	0.2383 ± 0.023
10	0.0561 ± 0.003	0.5718 ± 0.022	0.2859 ±0.036
20	0.0540 ± 0.003	0.6192 ± 0.025	0.2159 ± 0.008
40	0.0535 ± 0.001	0.5794 ± 0.004	0.2641 ± 0.008
60	0.0566 ± 0.003	0.5701 ± 0.021	0.2976 ± 0.044
Watermelon seed oil			
0	0.0520 ± 0.001	0.6041 ± 0.018	0.2325 ± 0.024
4	0.0490 ± 0.001	0.6166 ± 0.021	0.2043 ± 0.026
8	0.0525 ± 0.001	0.5973 ± 0.027	0.2280 ± 0.046
15	0.0495 ± 0.002	0.6330 ± 0.010	0.1851 ± 0.008
30	0.0484 ± 0.002	0.5794 ± 0.018	0.2450 ± 0.015
60	0.0510 ± 0.005	0.5339 ± 0.048	0.2822 ± 0.087

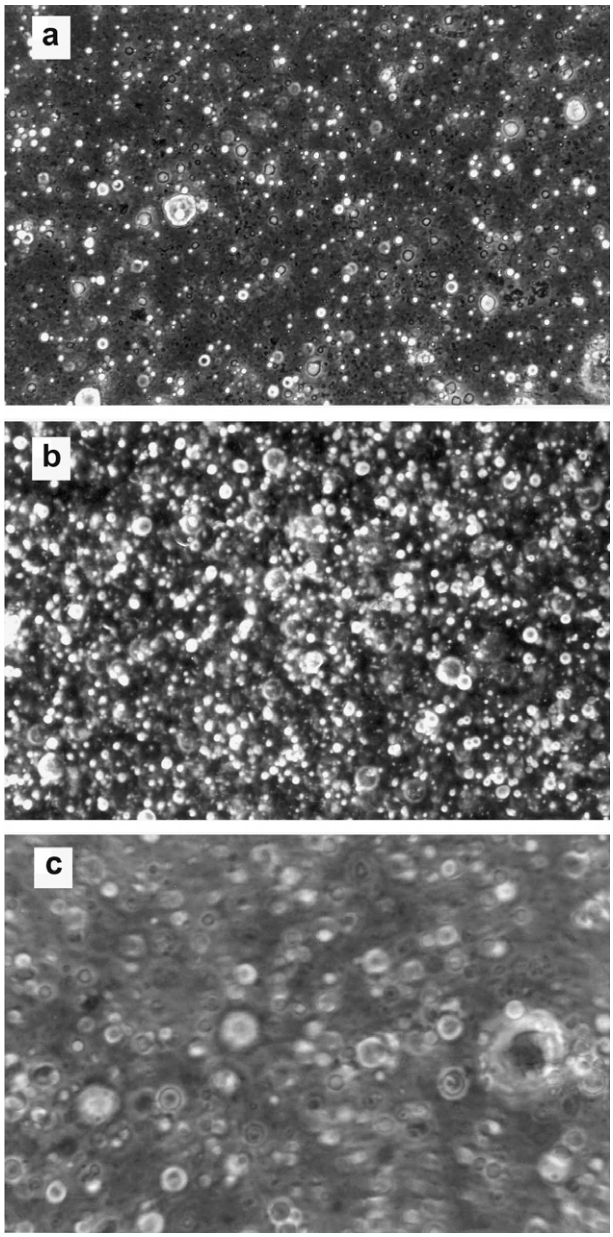

Fig. 3. Photomicrographs of avocado oil emulsions on different days of storage. (a) 1st day, (b) 30th day and (c) 60th day – under a constant magnification of 40× (scale: 1/10 i.e. one tenth of the photomicrograph's breadth is 24 μm.).

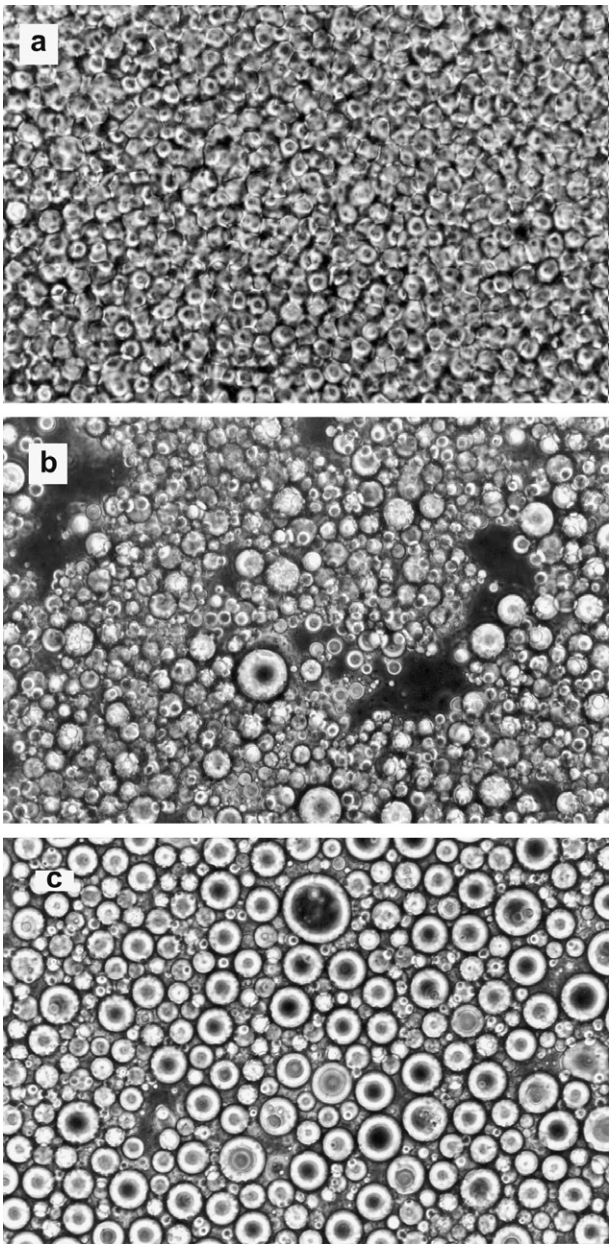

Fig. 4. Photomicrographs of watermelon oil emulsions on different days of storage. (a) 1st day, (b) 30th day and (c) 60th day – under a constant magnification of 40× (scale: 1/10 i.e. one tenth of the photomicrograph's breadth is 24 μm.).

slightly larger particles with diameters greater than 5 μm. The rheological behaviour, observed as apparent viscosity, was between 0.050 and 0.056 Pa s over the entire period of storage time. This meager increase in viscosity values may be an effect of the size increase of particles. In the case of watermelon oil-based emulsions (Fig. 4), about 90% of the particles were below 5 μm in diameter prior to storage. An increase in storage period increased the size of the particles (Table 3) though it hardly exceeded 10 μm.

The rheological parameters during storage did not significantly change the flow parameters, e.g. flow behaviour and consistency indices, and apparent viscosity (Table 2).

Table 3
Particle size[a] of emulsion droplets during storage

Range of particle size (μm)	Storage period (days)					
	Avocado emulsion			Watermelon emulsion		
	1	30	60	1	30	60
<5	100	100	70	90	85	70
5–10			30	10	15	30

[a] Values are expressed as percentage distribution of size.

Further, cream and serum separation of emulsions were not noticed during the storage period, meaning the absence of major structural deterioration of emulsion particles. The

overall conclusion is that the stabilities of these two emulsions are not affected during storage for up to 2 months at room temperature.

4. Discussion

The rheology of emulsions is influenced by several parameters, the most important being the inter-particle forces. In dilute emulsions, e.g. milk, the particles are far apart and the inter-particle interactions are relatively weaker. This emulsion possesses a low viscosity and is Newtonian in behaviour. In concentrated emulsions, e.g. mayonnaise, however, the particles are very close, resulting in strong inter-particle interactions to give a non-Newtonian behaviour (Rahalkar, 1992). It has also been reported that the shear-rate encountered in the human mouth for the consumption of soup is about 50 s^{-1}. The most speculative application of emulsion rheology is perhaps the development of novel food emulsions that also offer a health benefit.

Characterization of an emulsion, based on measurements employing coaxial cylinder systems, is the most common because of simplicity in measurement and ease in interpretation. An emulsion of lecithin in water or oil has been characterized and the flow parameters, such as consistency index and flow behaviour index have been reported (Bhattacharya, Shylaja, Manjunath, & Udaya Sankar, 1998). Shear-thinning non-Newtonian behaviour has been reported for such samples. This behaviour is expected as an increasing shear-rate disrupts the native structure of emulsion and aggregates, leading to a decrease in overall resistance to flow. Hence, shearing action during measurement of rheological properties affects the weak forces, such as hydrogen bonding and van der Waals force. The effect of viscosity on the texture perception of model dairy emulsions (using pectin and xanthan as thickening agents), and shear-thinning and non-Newtonian behaviour over a range of shear-rates were reported earlier (Relkin & Sourdet, 2005).

The binding of the particles in an emulsion is the result of three types of forces, e.g. attractive, repulsive and steric interactions. The most common attractive interaction is that of van der Waals which is universal in all food emulsions and it arises due to orientational, inductional and dispersion effects (Israelachvili & Ninham, 1977; Tabor & Winterton, 1969). The van der Waals interaction is long range in nature and is characterized by the Hamaker constant.

A small amount of van der Waals force is desirable as it leads to the formation of a network-like structure to stabilize the emulsion products. Strong van der Waals forces lead to the formation of individual aggregates rather than network structure, where the system destabilizes into two phases, i.e. aggregates and the surrounding fluid, and hence, becomes undesirable. In rheological terms, it leads to lower viscosity and sometimes even to a Newtonian behaviour. An example of this is in cheese making where the droplets in milk are in the form of oil-in-water emulsion, stabilized mainly by the milk proteins (Rahalkar, 1992). Mixing an acidic substance with milk lowers the pH and promotes stronger attractions. This leads to the formation of large aggregates, resulting in separation of milk into whey and solids. This also illustrates that inter-particle forces are strongly influenced by the presence of charge on the droplet and hence by the type and concentration of ions present in the surrounding medium.

In o/w emulsions, the amounts of fat and protein and the properties of the latter ingredient, and their particle sizes are expected to influence the rheological behaviour. The aggregation/coalescence of the droplets here plays a substantial role in their stability during storage. The values of different droplet sizes and their distribution patterns, as by phase contrast microscopy, are considered almost unimodal (i.e., between 2 and 10 μm). Such a narrow range of variations in the particle diameters may not markedly influence particle behaviour, and the incidence of aggregation, flocculation or coalescence is marginal. Hence, the variations of droplet sizes in this range possibly do not have any significant effect on the rheological status of these emulsions.

5. Conclusions

Marginal shear-thinning behaviour is exhibited by avocado pulp oil and watermelon seed oil with a range of flow behaviour index values between 0.86 and 0.88 while emulsions formed with these oils in the presence of WPC are pronouncedly non-Newtonian and shear-thinning. These emulsions, during storage at room temperature for up to 2 months do not show any significant change in their rheological status, and thus, can be considered as stable samples for use as food and nutraceutical delivery systems.

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