Colloidal Stability and Rheological Behaviour of Rapeseed Oil in Water Emulsions Stabilized with Whey Protein and Chitosan

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ABSTRACT

The impact of viscosity on emulsion stability at pH 2 using 1% (w/w) rapeseed oil (RSO) with 0.2 and 2% (w/w) whey protein concentrate (WPC) and 0.1 % (w/w) chitosan (CH) during 1 and 16 days of storage was investigated. The droplet size values obtained by image analysis were correlated with the viscosity values. All the emulsions behaved like non-Newtonian fluids, having shear-thickening characteristics, as the flow behaviour index values were larger than 1. A reduction in emulsion droplet size from 2.24 (2% WPC) to 0.95 μm (2% WPC, 0.1% CH) was observed when chitosan was added to the emulsions containing WPC (day 1). Addition of chitosan to WPC-emulsions caused about 20% relative increase in the viscosity. An increase in viscosity with a decrease in droplet size was observed. During the storage for up to 16 days, no water separation of WPC-chitosan emulsions was noticed, indicating that the stability of these emulsions was maintained at 4 ^{o}C. The stability observations were related to reduced droplet size and increased viscosity of the emulsions. The WPC and WPC-Chitosan stabilized emulsions with better shelf life at pH 2 have applications in drug delivery systems and pharmaceuticals.

INTRODUCTION

Proteins and polysaccharides are the most used biopolymers in food emulsions. Principally, proteins, due to their high surface activity, are important for the emulsion activity and stability, while polysaccharides acts as thickening or viscosifying agents[1,2]. The molecular weight of the components, pH, ionic strength, temperature of the emulsion and the molecular interactions play a role in emulsion formation and stability[3,4]. Different intermolecular forces that contribute to the protein-polysaccharide interactions are important in this respect[3].

Whey proteins are widely used in food emulsions due to their high emulsion activity[5,6]. Chitosan is a cationic polysaccharide with a pKa ~ 6.2-7.0[7,8]. It is prepared from crab and shrimp waste. As a dietary fibre CH has physiological and nutritional significance, good emulsifying properties and water/fat/dye binding capacity[9,10,11]. In acidic environments, CH is a good viscosity enhancing agent.

The utilization of emulsions in food products depends upon stability, viscosity and sensory perception. Hence, the understanding of the rheological characteristics of emulsions is necessary in product development for the stability during storage.

The amount of proteins and/or polysaccharides present in the emulsion is critical for the stability and in turn this influences the rheological status of the system. Emulsified fat can strengthen the viscosity and cream formation properties of the emulsion[12]. The number and size of oil droplets and their overall distribution pattern contributes substantially to the emulsion rheology[13]. Rheological characterization of food emulsions provides a better insight between the different components in the system[14].

There are few reports on WPC-CH stabilized emulsions and their rheological characteristics[15,16,17,18]. The rheological behaviour and stability of WPC-CH emulsions with low concentration (1%) of rapeseed oil has not yet been reported before.

In the current study, the storage-stability of the oil in water (o/w) emulsions containing WPC (0.2 and 2%), 0.1% CH and 1% RSO at pH 2 was examined using rheological characterization and microstructures.

MATERIALS AND METHODS

Chitosan with 75-85% de-acetylation and 200-800 cP viscosity was obtained from Sigma Aldrich (St Louis, MO). Whey protein concentrate with 80% protein content was obtained from Tine BA, Norway. Glycine was procured from Sigma Aldrich. Rapeseed oil (Askim Frukt and Bærpresseri AS, Norway) was purchased in a local super market.

Preparation of emulsions

Stock solutions of WPC (10 w/v %) and chitosan (1 w/v %) were prepared in 100 mM Glycine-HCl buffer, pH 2. The chitosan solution was left at 4 °C for one night before preparing the emulsions. Necessary concentrations (w/w %) of WPC, chitosan stock solutions and RSO were pre-mixed at pH 2 using an ultra-turrax (IKA yellow line DI25, Montebello Diagnostics, Norway) at 13000 rpm for 2 min. The emulsions were then homogenized by a continuous re-circulation (total eight cycles) in a M-120 E Micro-fluidizer (Microfluidics Int. Corp, Newton, USA) for 2 min and 8-10 kpsi pressure. During the micro-fluidization process the temperature of emulsions was maintained at 4 °C. 0.02% (w/v) sodium azide (NaN_3) was added as an antimicrobial agent to the emulsions before storage. All emulsions were prepared in two batches on different days before the analysis.

Emulsion stability during storage

20 mL of the emulsion was transferred to a glass stoppered bottle on day 0 and stored for 16 days at 4 °C prior to visual observations. The emulsions were observed for homogeneity/creaming/formation of water layer at the bottom from day 1 to 16 to check the extent of stability.

Rheological measurements

Flow curves of the emulsions were measured with a Physica MCR 301 rheometer (Par Physica, Anton Paar, Germany) together with a Titanium CC27 cup and a ST24-2D/2V/2V-30/129 stirrer. The stirrer was positioned with a 0.5 mm clearance from the bottom of cup. The temperature was controlled by a peltier and set to 20 °C. Viscosity curves were obtained in the range of $\gamma = 0.001 - 800\ s^{-1}$. All rheological measurements were conducted on triplicate samples.

Confocal laser scanning microscopy

A Leica TCS SP2 confocal laser scanning microscope (Leica Microsystems, Heidelberg, Germany) was used to take the emulsion images. The fat droplets in the emulsions were observed using CLSM. Samples of emulsion (1 mL) were stained with 0.1 mL of 1.0% (w/v) Nile Red. Each stained sample was placed on a concave confocal microscope slide (Sail; Sailing Medical-Lab Industries Co. Ltd., Suzhou, China), covered with a cover slip. The samples were then inverted for CLSM analysis after staining. Each sample (spot) was imaged in five places.

Droplet size distribution

Confocal micrographs were analysed for mean droplet size and cumulative size distribution using image processing software, ImageJ (Wayne Rasband, NIH, USA). The numbers of bins used for droplet size distribution histogram were specified as 100 during size distribution analysis and droplet count.

Statistical analysis

One-way ANOVA and 95% Tukey simultaneous confidence interval tests were performed.

Table 1 shows the composition of WPC, chitosan and RSO used in emulsion preparation.

Table 1. Composition (w/w %) of the different emulsions at pH 2.

Emulsion No.	Chitosan	WPC	RSO
1	0	0.2	1
2	0.1	0	1
3	0	2	1
4	0.1	0.2	1
5	0.1	2	1

RESULTS

On day 1 during storage all emulsions except no. 2 (CH emulsion) appeared to be homogeneous (Fig. 1). Emulsion no. 2 with 0.1% CH, separated in to two phases, a thin oily layer on top, indicating coalescence. Appearance of a watery layer at the bottom of emulsion no.1 and no. 3 (WPC 0.2% and 2%) on day 16 was noticed, indicating a slow reduction in emulsion stability during storage. The emulsions containing both WPC and CH seemed to be stable during storage until 16 days (Fig.1).

Rheograms of emulsions are represented in Fig. 2. The emulsions during storage on days 1 and 16 behaved like non-Newtonian fluids, having shear-thickening characteristics. The flow behaviour indices were higher than 1. The shear rate/shear stress data could be adequately fitted to the common rheological equation, i.e. power law model. Table 2 shows viscosity values obtained for the different emulsions during 1 and 16 days of storage.

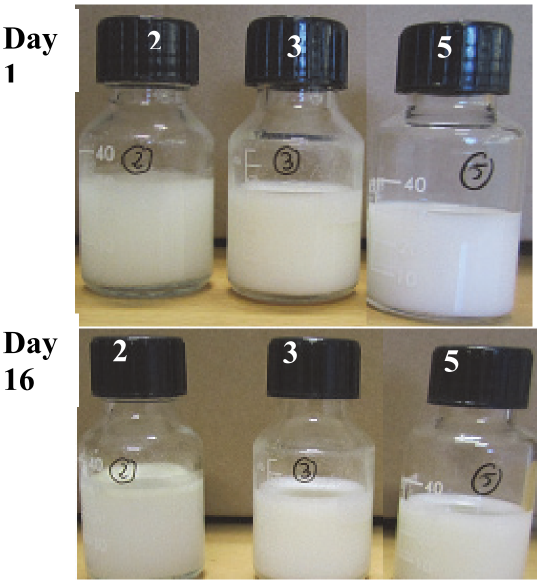


Figure 1. Photographs of WPC-chitosan emulsions during storage, day 1 and day 16 at pH 2. Number indicates Emulsion No. in Table 1.

Emulsions prepared without chitosan showed low viscosity during storage. An increase in the viscosity values when chitosan was added to all WPC emulsions was shown. The viscosity values decreased on day 16 as compared to day 1.

Fig. 3 illustrates the confocal micrographs of emulsions. The emulsions stabilized by 0.2% WPC had homogeneously distributed individual oil droplets with no sign of flocculation on day 1.

With the addition of 0.1% CH, smaller sized droplets were observed (Fig. 3A and B). Larger oil droplets were observed in the emulsion containing 0.1% CH alone (data not shown). The emulsions containing 0.1% CH, 2% WPC have smaller droplets as compared to the WPC emulsion without added CH (data not shown).

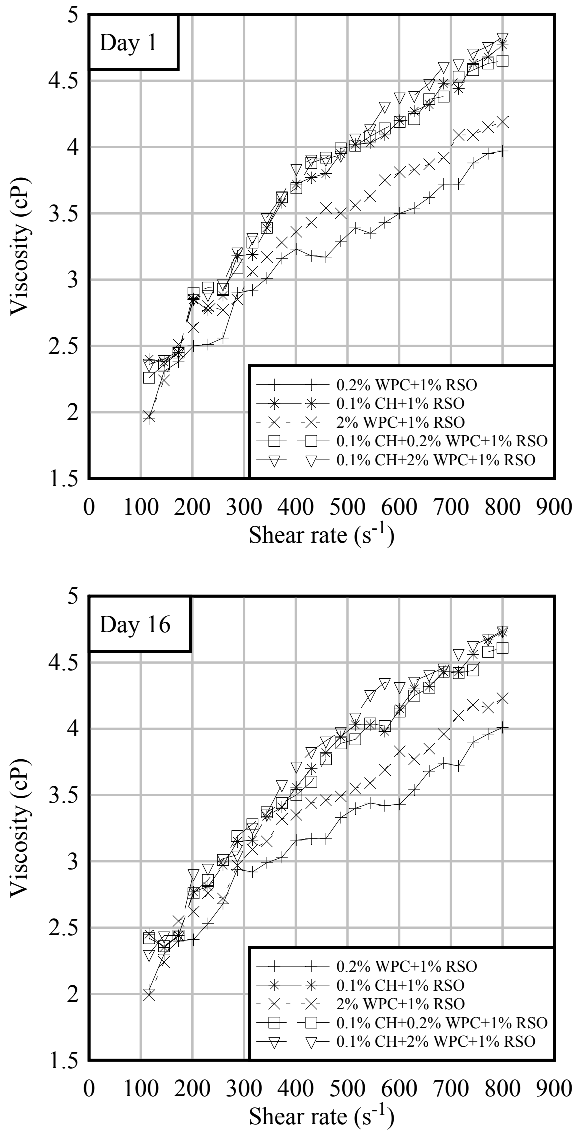


Figure 2. Rheograms of WPC-CH emulsions on day 1 and day 16 during storage at pH 2.

Size of the droplets was analysed from the CLSM images of emulsions (Fig. 4). D10 values calculated from the size distribution data during storage on 1 and 16 days are shown in Table 3.

The droplet size (D10) values were reduced with the addition of CH to the WPC emulsion (Table 3). An increase in the droplet size during storage from day 1 to 16 (WPC+CH emulsions) was also evident. Comparing Tables 2 and 3, it is clear that the viscosity increased with a decrease in D10 values during 1 and 16 days of storage. (One-way ANOVA and 95% Tukey simultaneous confidence interval test result).

Table 2. Viscosity values (η) of the different emulsions.

No.	Chitosan (w/w %)	WPC (w/w %)	Oil (w/w %)	η (cP)at γ=800 /s	
				Day 1	Day 16
1	0	0.2	1	3.97	4.01
2	0.1	0	1	4.77	4.70
3	0	2	1	4.20	4.20
4	0.1	0.2	1	4.65	4.60
5	0.1	2	1	4.83	4.70

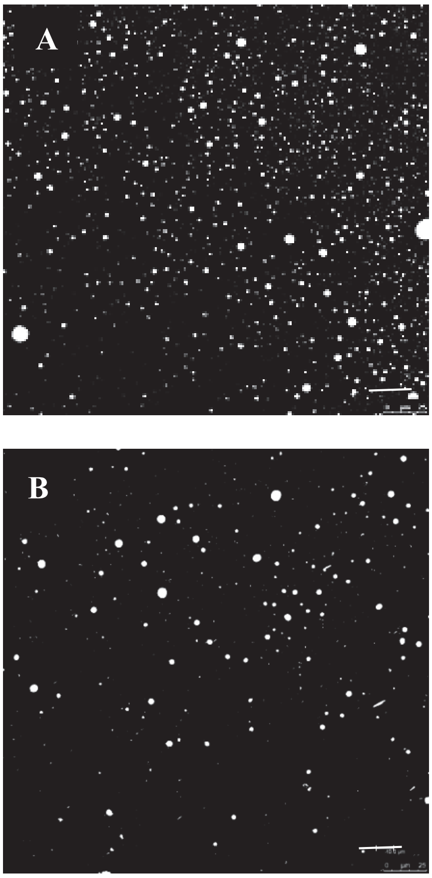
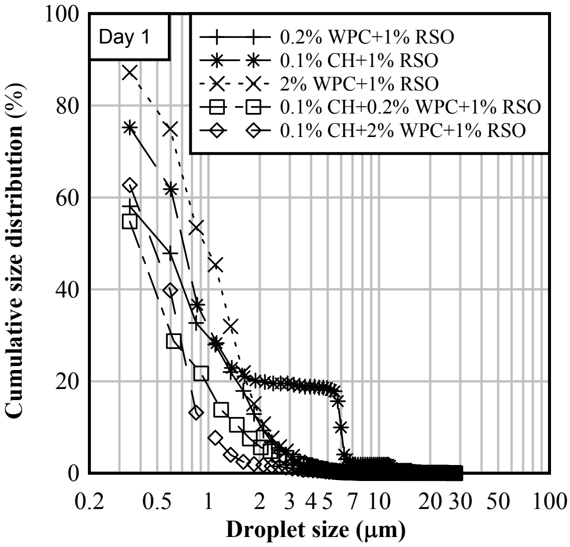


Figure 3. Confocal micrographs of o/w emulsions during storage day 1. Scale bars are 20 μm.
A. 0.2% WPC+1 % RSO,
B. 0.1% CH+0.2% WPC+1% RSO.

The 2% WPC emulsion showed 5% relative increase in viscosity with respect to the 0.2% WPC emulsion (day 1).



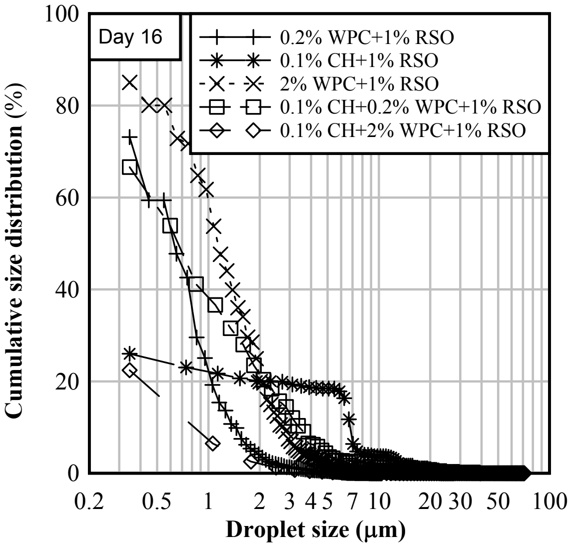


Figure 4. Size distribution plot of WPC-CH emulsions on day 1 and day 16 during storage at pH 2.

However, when 0.1% CH was added the relative increase in viscosity was about 20%. Overall, emulsions with 0.1% CH and 2% WPC showed greater viscosity.

DISCUSSION

The pI of WPC ~ 5.1 and that of CH is ~ 6.5. At pH 2.0, both WPC and CH have positive charges. Earlier reports on WPC-CH emulsion system have showed that the formation of soluble or insoluble complexes may be pH dependent[15,16,17]. In these systems they suggest that the electrostatic interaction is the mechanism involved in WPC-CH emulsion at pH>5 and higher oil concentration (10 v/v %). However, another study reported that the thermodynamic incompatibility might be the mechanism of WPC-CH stabilized emulsions (40% RSO) at pH 3[18].

Table 3. Droplet size (D10) values of emulsions during storage on day 1 and 16 at pH 2.

*No.	D10 (μm)	
	Day 1	Day 16
1	2.06	1.40
2	Phase separation (unstable)	Phase separation (unstable)
3	2.24	2.72
4	1.44	3.34
5	0.95	1.04

*Emulsion numbers: composition of WPC, CH, RSO and respective viscosity (η) values are shown in Table 2.

In the present study, WPC-CH emulsions prepared with 1% RSO at pH 2 were stable after 16 days of storage. The mixture of WPC-CH system seems to have larger surface area than WPC emulsions.

Image microscopy results showed that CH addition to WPC emulsions reduced the droplet sizes. The CH molecules have a larger effect in increasing viscosity of the aqueous phase of emulsions than the whey proteins. Since the CH molecule is much larger in size and more extended as compared to whey proteins, the CH molecule occupies greater hydrodynamic volumes and hence a higher viscosity emulsion is obtained. A reduction in droplet size also showed increase in emulsion viscosity. The observed increase in viscosity could be due to the decrease in mean droplet size and polydispersity of the emulsions[19]. This also depends on the molecular weight, degree of acetylation and the concentration of CH used in WPC emulsions. For instance, a

2% WPC emulsion together with 0.1% CH showed higher viscosity and stability during storage from day 1 to 16. During the storage for up to 16 days, no water separation of WPC-chitosan emulsions was noticed, indicating that the stability of these emulsions was maintained at 4 °C. These observations were in agreement with emulsion droplet size and viscosity.

The WPC-CH stable emulsions can be considered for utilization in acidified milk products and food or neutraceutical delivery systems at low pH.

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