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# Emulsions stabilized by nanofibers from bacterial cellulose: new potential food-grade Pickering emulsions

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## Abstract

In the present work, we investigated the formation and stability of Pickering emulsions stabilized by nanoparticles generated from bacterial cellulose (BC) by hydrochloric acid hydrolysis. The resulting particles, called nanofibers, presented a ribbonlike shape with diameters of 30-80 nm and range in length from 100 nm to several micrometers. The obtained nanofibers showed good hydrophilic and lipophilic properties and had significant ability to reduce the surface tension of oil/water droplets from 48.55 ± 0.03 to 34.52 ± 0.05 mN/m. The oil-in-water Pickering emulsions with a peanut oil concentration of 15% (v/v) were stabilized by only 0.05% (w/v) nanofibers and displayed a narrow droplet size distribution and high intensity with an average droplet size of 15.00 ± 0.82 nm. The morphological studies confirmed the nano-scaled droplets of emulsions. The effects of pH values and temperatures on the creaming ability and physical stability were also evaluated by zeta-potential and droplet sizes. Results showed that emulsions displayed relatively lower creaming ability at pH < 7, while displayed optimal physical stability and dispersibility at pH ≥ 7. The temperature (20-100 °C) and time-dependent test (0-4 weeks) indicated that the Pickering emulsions stabilized by only 0.05% (w/v) nanofibers displayed excellent stability. Due to the sustainability and good bio-compatibility of nanofibers from BC, the developed emulsions stabilized by low concentration of nanofibers can be used as new food-grade Pickering emulsions and have great potential to deliver lipophilic bioactive substances in food industry.

**Keywords:** Bacterial cellulose; Nanofibers; Solid particles; Pickering emulsions; Physical stability

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## Introduction

An emulsion is a dispersed heterogeneous system composed of two or more completely or partially immiscible liquids, where one of the liquid phases (the dispersed phase) is distributed in the other (the continuous phase) (Dickinson, 2015). Stabilizations of emulsion droplets are conventionally conducted by either reducing interfacial tension via small molecular weight surfactants or forming steric interfacial films through proteins and hydrocolloids (Aveyard et al., 2002; Tavernier, Wijaya, Van der Meeren, Dewettinck, & Patel, 2016). In the past few years, stabilization of emulsion droplets by solid particles is another approach that has received increasing attention, which is referred as Pickering emulsions (Tavernier et al., 2016). By definition, Pickering emulsions are solid particle-stabilized emulsions in the absence of any molecular surfactant, where solid particles are adsorbed to an oil-water interface. Compared to the conventional emulsions, Pickering emulsions have notable ability to stabilize phase interfaces against Ostwald ripening and coalescence because of the micro- or nanometer sized particles adsorbing irreversibly at interfaces (Wei, Wang, Zou, Liu, & Tong, 2012; Wooster, Golding, & Sanguansri, 2008). The irreversible adsorption at oil-water interfaces, caused by capillary energy, is several orders of magnitude higher than thermal energy. Therefore, it is widely accepted that micro- or nanoparticles have offered distinct advantages in various fields, especially for delivery of bioactives, ranging from improving stability to controlling the release and targeting of the bioactive for enhanced functionality in food and medical science (Yao et al., 2013). For the increasing interest in the use of dispersed particles, much of

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the fundamental research has been focused on the study of inorganic particles (especially silica particles), or concerned with their chemical modification particles (Binks & Tyowua, 2016; Tyowua, Yiase, & Binks, 2017; van Wijk, Salari, Zaquen, Meuldijk, & Klumperman, 2013). However, as a food additive, safety of the inorganic nanoparticles is necessary to be considered. Therefore, a critical ongoing challenge is to be able to produce macro- or nanoparticles that are both effective as Pickering stabilizers and also acceptable for use in food products on the commercial scale.

Recently, there has been increasing interest in the utilization of surface-active polysaccharides as emulsifiers including pectin, gum arabic, modified starches, modified cellulose and galactomannans due to their excellent particle hydrophobicity, high adsorption capacity, biodegradability and biocompatibility (Sarika, Pavithran, & James, 2015; Tu et al, 2017; Yusoff & Murray, 2011; Zhang et al, 2015). Among them, cellulose has widely been applied in cosmetics or pharmaceuticals as stable matrix for sufficient interfacial activity (Dickinson, 2012; Do, Mitchell, Wolf, & Vieira, 2010; Singh, Kaushik, & Ahuja, 2016; Wang et al., 2016). However, many of them are confined to specific food products due to their modification which further complicates issues on altering their bio-based character, biodegradability and high cost (Do et al., 2010; Winuprasith & Suphantharika, 2013). Therefore, it is critical to explore low cost and better bio-compatibility nanoparticles for Pickering emulsions in food manufactures.

Bacterial cellulose (BC), an extremely pure natural microbe exocellular polysaccharide, can form hydrogels with better material properties including high

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purity, high crystallinity, high degree of porosity, high water-uptake capacity, high tensile strength, excellent bio-compatibility and 3D nanofibrillar cellulosic network properties (Lin, Lopez-Sanchez, & Gidley, 2015, 2016; Römling & Galperin, 2015). Therefore, BC represents a potential alternative to plant-derived cellulose for specific applications in bio-medicine, cosmetics, high-end acoustic diaphragms, papermaking, conductive polymers and other applications (Chen et al., 2013; Mohamad, Amin, Pandey, Ahmad, & Rajab, 2014; Silva et al., 2014; Tian et al, 2017; Ul-Islam, Khan, & Park, 2012). In addition, BC, a type of dietary fiber, is well known as traditional gel delicacy (nata) in the Southeast Asia (Phisalaphong et al., 2016; Piadozo, 2016), and is classified as generally recognized as safe (GRAS) by the USA Food and Drug Administration in 1992 (Rana et al., 2014). Due to its suspending, thickening, water-holding, stabilizing, bulking and fluid properties, BC has been demonstrated as a promising low calorie bulking ingredient for the development of novel rich functional foods of different forms such as powder, gelatinous or shred foams (Rana et al., 2014). Recently, it has been showed that natural nanocrystals or nanowhiskers from BC or with grafted are promising potential emulsifiers in petrochemical industry (Irina Kalashnikova, Bizot, Cathala, & Capron, 2011; Lee, Blaker, Murakami, Heng, & Bismarck, 2014). However, few studies have shown that BC or BC ramification can be used as stabilizer in oil-in-water system in food industry (Paximada, Koutinas, Scholten, & Mandala, 2016). There is still little information about the nanofibers from BC used as Pickering stabilizer in oil-in-water system in food industry. In order to explore the new food-grade nanoparticles for food emulsions, it is necessary to

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understand the properties of the Pickering emulsion stabilized by nanofibers from BC and its stabilized mechanism involved in the oil-in-water system.

Hence, the objective of this research was to investigate the properties of food-grade Pickering emulsions stabilized by nanofibers. Nanofibers were obtained from bacterial cellulose by hydrochloric acid hydrolysis and were characterized by TEM, contact angle and surface intension tests. The resulting nanofibers were used as stabilizer in peanut oil/water system, and their emulsifying capacity were evaluated. The effects of pH, temperature and time-dependence on the stability of the prepared emulsions were investigated and their possible stability mechanisms were also discussed.

## 2. Materials and methods

### 2.1 The cultivation of bacterial cellulose

Bacterial cellulose was cultured with our previous method. In brief, the *Komagataeibacter xylinus* (formerly *Gluconacetobacter xylinus*) strain CICC 10529 (China Centre of Industrial Culture Collection, Beijing, China,) was cultured at 30 °C under static conditions in fermentation medium (pH 5.0) containing glucose 2% (w/v), yeast extract 0.5% (w/v), $K_2HPO_4$ 0.1% (w/v), $MgSO_4$ 1.5% (w/v), ethanol 2% (v/v) (Lin, Li, Lopez-Sanchez, & Li, 2015). After 14 days cultivation, cellulose membranes were harvested and rinsed with running water overnight, soaked in 0.1 M NaOH solution at 80 °C for 2 h, and then washed with deionized water several times to completely remove alkali. The purified cellulose membranes were kept in the sterilized water and stored at 4 °C in the fridge (Wu et al., 2010).

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### 2.2 The preparation of nanofibers

Nanofibers were prepared according to the method in literature with some modifications (Martinez-Sanz, Lopez-Rubio, & Lagaron, 2012). Briefly, the purified wet BC membranes were disrupted in a blender (CWFJ, Changzhou, China) at 15000 rpm for 5 min at room temperature. Then the aqueous cellulose suspensions were centrifuged (3040 *g*) for 10 min to remove the free water, followed by hydrolysis with 2.5 M HCl at 70 °C under magnetic stirring condition for 1 h (Kalashnikova, Bizot, Cathala, & Capron, 2012). After hydrolysis, the suspensions were washed with deionized water and centrifuged at 10000 *g* until its pH is neutral. Nanofiber suspensions were stored at 4 °C in the fridge for further study.

### 2.3 Contact angle measurement

The contact angle of nanofibers was measured using an OCA 20 AMP (Dataphysics Instruments GmbH, Germany). Nanofibers were prepared as films of 13 mm in diameter and 1-2 mm in thickness with membrane plate, and the films were placed into an optical glass cuvette containing purified corn oil. Then, a drop of Milli-Q water (4 μL) or peanut oil (4 μL) was deposited on the surface of the film using a high-precision injector. After 4 min for equilibration, the drop image was photographed using a high-speed video camera, and the profile of the droplet was numerically solved and fitted to the Laplace-Young equation. Three measurements were performed for each sample.

### 2.4 Preparation of Pickering emulsion

Two sets of Pickering emulsion were prepared. In the first set, peanut oil/water

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emulsions were prepared using 10 mM phosphate buffer solution (PBS, pH=7.0) to reach an oil-in-water ratio of 15% (v/v), where the final concentrations of nanofibers were 0.01, 0.03, 0.05, 0.07, and 0.09% (w/v), respectively. In the second set, peanut oil/water with different oil-in-water ratios (5, 10, 15, 20, 25, 30%, v/v) was prepared with PBS (10 mM, pH = 7.0), where the concentration of nanofibers was a constant value of 0.05% (w/v). All above samples were mixed in a high-shear blender (F6/10-10G, Fluco, Germany) at 15000 rpm for 1 min, and followed by homogenization for 1 min with the high-pressure homogenization (Panda Plus, Parma, Italy) at 600 bar.

**2.5 Surface tension test of droplets**

The interfacial tension of droplets was measured at 25 ± 0.5 °C according to the Du Nouy ring-pull method using a surface/interface digital tensiometer (DCAT 21, Dataphysics Co., Germany) equipped with a standard ring probe and an SV 20 glass vessel. A platinum ring was rinsed with ultrapure water and acetone, and then re-rinsed with ultrapure water, followed by passing through a blue flame and cooled before each measurement. 20 g of the peanut oil phase was carefully layered on top of 30 g of the aqueous phase and left for 30 min at 25 °C before each test. The maximum force needed to pull the ring from one phase to another is equal to the interfacial tension between the two immiscible liquids. Each sample was analyzed three times, and the data are reported as the average. The standard deviation of the interfacial measurements was lower than 0.2 mN/m.

**2.6 Transmission electron microscopy**

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The morphologies of natural bacterial cellulose suspensions, nanofibers suspensions and emulsions were characterized using a HT-7700 transmission electron microscope (Hitachi Ltd., Japan). 10 μL of samples diluted with distilled water (1:1000) were deposited on a freshly glow-discharged carbon-coated electron microscope grid (200 mesh copper, Delta Microscopies, France) and the excess was removed by filter paper, and then the grids were dried at room temperature (25 °C). For the emulsions, negative staining was performed by adding 10 μL of 2% phosphotungstic acid solution (w/v), followed by removing the excess solution after 10 min staining, and then the grids were dried at room temperature (25 °C). Finally, the grids were observed under standard conditions with a HT-7700 transmission electron microscope operating at 80 kV. A Quemesa CCD camera and TEM image analysis software (Gatan Digital Micrograph, US) were used in the capturing and processing images of the nanofibers. And the width and length were measured from 50 individual nanofibers.

**2.7 Droplet size and zeta-(ζ) potential measurements**

Droplet size distributions of all emulsions were measured using a Malvern particle size analyzer (Nano ZS90, Malvern Instruments Ltd., UK) equipped with Zetasizer software (Version 7.11). The droplet size was then determined by dynamic light scattering (DLS) with same equipment. The refractive index (RI) of sample was 1.590 with an absorption index (AI) of 0.010, and RI of the continuous phase was 1.330. Measurements were made at a scattering angle of 90° at 25 °C, and the diffusion coefficients and hydrodynamic diameters of the droplets were calculated

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according the method of previous report (Mikulcova, Bordes, & Kasparkova, 2016). The droplet sizes were also expressed as the volume-length mean particle diameter ($d_{4,3}$), which was calculated from the number of particles ($n_i$) with the corresponding diameter ($d_i$) based on the following equation:

$$d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (1)$$

Where, $n_i$ is the number of particles with the same diameter; $d_i$ is the particle size.

The zeta potentials of Pickering emulsions were measured using a Nano-Zeta Potential Analyzer (Malvern Instruments Ltd., UK). In briefly, all samples were diluted to 0.01% (v/v) using PBS, and 0.5 mL of diluted emulsion was injected into measurement cell and then the electrophoretic mobility of the particles was measured. RI and AI of sample were set as 1.590 and 0.010, respectively. The dielectric constant was set as 78.5. Three replicates were tested for each sample.

**2.8 Effects of pH, temperature and storage time on the emulsion stability**

The effects of pH, temperature and storage time on the stability of Pickering emulsions prepared with 20% peanut oil and 0.05% nanofibers were examined by the evaluation of droplet sizes and zeta potential. The pH stability test was performed by adjusting pH of the emulsions at 3, 5, 7, 9 and 11 using 0.1 M HCl solution or 0.1 M NaOH solution. The temperature test was carried out by heating the emulsions at 20, 40, 60, 80, 100 °C for 30 min respectively. For the time-dependent test, the stability of emulsions was evaluated under condition of storage for 4 weeks at room temperature (20 °C).

**2.9 Statistical analysis**

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All the experiments in this study were carried out using at least two freshly prepared samples. The statistical analysis was performed by analysis of variance (ANOVA) and test of least significant difference (Duncan's multiple-range, $p < 0.05$) using SPSS version 21. All data were expressed as means ± SD.

## 3. Results and discussion

### 3.1 Properties of nanofibers

It has been demonstrated that stable cellulosic nanoparticle suspensions can be prepared by treating native cellulose with harsh acid hydrolysis (Kalashnikova et al., 2012; Lee et al., 2014). In this study, nanofibers were obtained from bacterial cellulose with acid hydrolysis. Microstructures of bacterial cellulose before and after hydrolysis were observed by transmission electron microscopy (TEM). As shown in Fig. 1A, bacterial cellulose suspensions without hydrolysis displayed a delaminated and entangled micro-fibrils network and appeared irregular reticulate fibril arrangements, in agreement with the TEM images in the previous literatures (Lee et al., 2014). However, after hydrolysis it can be observed that there was more random dispersion of small micro-fibrils with diameters of 30-80 nm and range in length from 100 nm to several micrometers, indicating that the hydrogen bonds of the micro-fibrils network were disrupted by hydrolysis (Fig. 1B and C). It is well known that fibrils are composed of cello-silk by hydrogen bonds and intertwined with each other, so it can be deduced that microcrystalline cellulose peeled off from fibrils as result of the disrupted hydrogen bonds by hydrolysis, which was consistent with previous studies (Khattak et al., 2015; Lee et al., 2014). However, it could also notice

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that some larger micro-fibrils appeared in Fig. 1B, which was probably due to the fact that some micro-fibrils rearranged to a delaminated and entangled network again during processing (Lin, Li, Lopez-Sanchez, & Li, 2015; Paakko et al., 2007).

In order to further study the properties of nanofibers, contact angle (CA) was performed to study its hydrophilic and lipophilic properties by depositing a water droplet and an oil droplet directly at the top surface of a material as prepared, respectively. As depicted in Fig. 2A and B, the nanofibers were displayed CAs of 64.46 ± 3.56° and 27.29 ± 4.74°, respectively. It has been reported that materials with contact angle of 0-90° can be wetted by water or oil drop, exhibiting hydrophilic and lipophilic properties. Therefore, the present results showed that the nanofibers prepared here had both hydrophilic and lipophilic properties (Dickinson, 2009; Xiao, Li, & Huang, 2016).

**3.2 Dispersed properties of emulsions**

The above prepared nanofibers were added to the peanut oil/water system. After treatment by high pressure homogenizations there was dispersed and homogeneous emulsions present (Fig. S1), suggesting that the nanofibers were able to stabilize peanut oil/water interfaces against Ostwald ripening and coalescence. As described in Fig. 3A and Fig. 3B, the interface tension of only oil/water system was obviously larger (48.52 ± 0.03 mN/m) than that of emulsion system stabilized by nanofibers (34.52 ± 0.05 mN/m), indicating that hydrophilic moieties of nanofibers could bind with water and lipophilic moieties of nanofibers can absorb the oil, so it can absorb on the interfaces of oil/water to reduce the surface tension droplets, which also confirmed

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the above described result of contact angle.

In order to understand the emulsifying capacity of nanofibers prepared here, two sets of experiments were performed: one set was peanut oil/water emulsions prepared with different concentrations of nanofibers (0.01, 0.03, 0.05, 0.07, 0.09%, w/v) and an oil concentration of 15% (v/v), and a second set was peanut oil/water emulsion prepared with different oil concentrations (5, 10, 15, 20, 25, 30%, v/v) and a concentration of 0.05% (w/v) nanofibers. The appearance and droplet size distributions are presented in Fig. 4. Appearance of all emulsions stabilized by various nanofiber concentrations displayed good dispersed properties (Fig. 4A), while the droplet size distributions and intensity widened and decreased respectively with the increase of nanofiber concentrations (Fig. 4C). Even two peaks appeared in the emulsions stabilized by 0.07% (w/v) and 0.09% (w/v) nanofibers. This was probably due to the relatively high concentrations of nanofibers leading to the aggregating on the surface of the oil-in-water droplets as a result of the particle growth (>100 nm). It has been reported that linear polysaccharides have large space occupancy and a lower phase separation threshold concentration due to the entropy of mixing (excluded volume effect), and thus the relatively higher biopolymer concentrations would lead to the aggregating (Mao et al., 2013; Tolstoguzov, 2003, Dickinson, 2017). Therefore, 0.05% (w/v) nanofibers were selected to prepare Pickering emulsions for the further studies.

As shown in Fig. 4B, all emulsions presented excellent dispersed properties, except for emulsions prepared at the highest oil concentration of 30% (v/v). Some oil

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indicated by the red arrow was presented on the top of emulsion, which was probably due to the fact that low concentration of nanofibers could not absorb the peanut oil droplets completely. Fig. 4D described their corresponding droplet size distributions, and there was a wide distribution with low intensity when adding 5% (v/v) peanut oil in the system, which was probably due to the Ostwald ripening of droplets leading to the increase of droplet size. However, the droplet size distributions were sharply narrowed with an increase in oil concentrations, suggesting that the emulsions displayed good stability. It was also observed that the intensity tended to the slight decrease with the increase of oil concentrations, which would be caused by that more oil cannot be absorbed by nanofibers and then was separated out. Previous work reported that the particles might become directly attached to the oil-water interface, generating a particle-loaded surface layer which acted to protect individual droplets or bubbles against instability events such as droplet coalescence or bubble shrinkage.

**3.3 Morphology of Pickering emulsion**

The morphology of emulsion droplets was studied for dispersions prepared with 15% (v/v) peanut oil and 0.05% (w/v) nanofibers in PBS (pH=7.0) using TEM. It could be observed that there were many spherical particles with size of 10~30 nm as shown in Fig. 5, which was in agreement with the results obtained from the droplet sizes measurement described above. However, there were also some big droplets (＞ 30 nm) present, and this phenomenon might due to droplet aggregation during sample preparations for TEM observation. The droplet sizes of the emulsions prepared here were nano-scaled and were obviously smaller than those reported in the literature

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(Paximada, Tsouko, Kopsahelis, Koutinas, & Mandala, 2016), which was probably related to the present emulsions prepared with high-pressure homogenization treatment, as well as the nano-scaled stabilizer of the bacterial cellulose hydrolyzed by hydrochloric acid, while in the literature bacterial cellulose stabilizers were micro-scaled as a result of the mechanical treatment (Paximada, Tsouko, et al., 2016).

### 3.4 Effects of pH and temperature on dispersion properties of Pickering emulsion

pH value is considered as a basic parameter in the determination of biopolymer complexation due to its crucial role in the degree of ionization of the side functional groups carried by the biopolymers (Abdolmaleki, Mohammadifar, Mohammadi, Fadavi, & Meybodi, 2016). Therefore, in the present study, we evaluated the influence of pH on the dispersion properties of Pickering emulsion containing 0.05% (w/v) nanofibers and 15% (v/v) peanut oil. The appearance of emulsions prepared at pH values of 3~11 was shown in Fig. 6A, there was obvious creaming present in emulsions prepared at pH 3 and 5, while for the emulsions prepared at pH 7, 9 and 11, there was no lamination and all were stable and homogenized. As described in Fig. 6B, the $d_{4,3}$ of emulsions prepared at pH 3 and 5 were significantly larger than those of emulsions prepared at pH 7, 9 and 11, which was probably due to the formation of relatively large droplets population at the lower pH values, indicating that the coalescence was promoted at low pH. This phenomenon can be attributed to the relatively low net droplet charge at low pH range and therefore relatively weak electrostatic repulsion between the droplets. All the droplet size distributions became

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narrow and their corresponding intensity almost reached at 30% or even higher (data not shown), suggesting that the emulsions prepared here were very homogeneous. The effect of pH changing on the stability of Pickering emulsion may cause the change of the charges on the surface of droplets, which might lead to the above result (Mao et al., 2013), in accordance with the previous reports (Ngai, Behrens, & Auweter, 2005; Wei, Yang, Yang, & Wang, 2012). Since the emulsions in the present work would be used as food-grade emulsions, pH 7 was selected for further studies.

A large amount of researches have reported that emulsions have thermodynamic unstability and kinetic stability, which can easily destabilization under unfavourable environmental conditions (Jourdain, Schmitt, Leser, Murray, & Dickinson, 2009). In this regard, we investigated the effects of temperatures on the stability of Pickering emulsions in terms of droplet sizes. When the Pickering emulsions prepared at pH 7 were heated for 30 min at 20, 40, 60 and 80 °C, respectively, no obvious changes in their appearance was observed in all emulsions as shown in Fig. 6C. However, some oil leakage and oil layer formation was observed on the top of emulsions, when the emulsions were heated at 100 °C (indicated by a red arrow in Fig. 6C). Moreover, there were no significant difference in $d_{4,3}$ independently of the temperatures ($p > 0.05$) except for the emulsions at 100 °C (Fig. 6D), indicating that the droplet size of all emulsions did not change with the increase in temperatures. These results suggested that nanofibers had good ability to prevent droplets against aggregation over a wide range of thermal treatment conditions, which is in accord with the reported in literatures regarding that Pickering emulsions have notable ability to stabilize phase

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interfaces against Ostwald ripening and coalescence compared to normal emulsions (Dickinson, 2009; Mao et al., 2013).

**3.5 Zeta-potential**

Zeta potential is the electric potential in the interfacial double layer at the location of particle interface, which is a key indicator of the stability of colloidal dispersions (Cheong, Tan, Tan, & Nyam, 2016). Zeta-potential of emulsions prepared at various oil concentrations, nanofiber concentrations, pH values and temperatures are shown in Fig. 7. It was found that ζ-Potential of all emulsions was high enough, with a negative value at various concentrations of nanofibers and oil, and various temperatures (Fig. 7A, B and D). This result indicated that there was enough repulsive force among emulsion droplets to keep droplets away from each other. It has been reported that the absolute value of zeta potential more than 30 can confer stability in which the solution or dispersion will resist aggregation, whereas attractive forces will enhance and exceed this repulsion and the dispersion may break and flocculate when the potential is small (Hanaor, Michelazzi, Leonelli, & Sorrell, 2012; O'Brien, Midmore, Lamb, & Hunter, 1990). In this regard, the emulsion showed good stability. However, pH had significant effect on the ζ-Potential. It could be observed that zeta-potential values were less than -20 mV when pH was 3 and 5 (Fig. 7C), indicating an incipient instability for both acidic system emulsions, which was probably due to the increase of hydrogen ion resulting in a sharp increase of ζ-potential, and this finding was in agreement with the results presented in Fig. 6A. Moreover, the emulsions at pH 7, 9 and 11 all displayed good stability, suggesting that a neutral or alkaline system is optimal for the stability of emulsions. It is very likely that many of carboxyl groups in

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nanofibers can be ionized in neutral or alkaline system, and the nanofibers are mutual repulsed for more steric hindrance (Tu et al, 2017; Zhang et al, 2015)

### 3.6 Time-dependent behavior of Pickering emulsion at neutral pH

The time-dependent behavior of emulsions prepared 15% (v/v) peanut oil and 0.05% (w/v) nanofibers in PBS at pH 7 was evaluated by measuring droplet sizes weekly for 4 weeks. As shown in Fig. 8A, there were no significant changes in droplet size distributions, and all emulsions displayed narrow droplet size distributions with high intensity, suggesting that the nanofibers prepared here were excellent stabilizers for Pickering emulsions. Furthermore, $d_{4,3}$ of all emulsions was around 15~20 nm during storage time (Fig. 8B), displaying no markedly changes, in agreement with the results of droplet size distributions described above. In conclusion, the Pickering emulsions prepared here had a very slow Ostwald ripening rate and showed excellent stability.

## 4. Conclusions

Results from the present work showed a simple method for the formation and stability of Pickering emulsions using nanofibers from bacterial cellulose (BC). Nanofibers, generated by 2.5 M HCl hydrolysis, displayed the characteristic of nano-scaled cello-silk, and showed good hydrophilic and lipophilic properties, and exhibited significant effect on reducing the surface tension of oil/water droplets. Only 0.05% (v/v) nanofibers were able to stabilize oi-in-water system at neutral pH and displayed a narrow droplet size distribution with high intensity, nano-scaled diameter size and homogenous emulsions. Low pH values had significant effect on the stability of emulsions, while emulsions displayed optimal stability and dispersibility when pH

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≥ 7. The temperature and time-dependent test indicated that the present Pickering emulsions had relatively high stability at the selected temperature and time range. However, determination of the mechanisms of nanofibers working as a good emulsifier requires further studies, and this work provides knowledge not only for understanding the formation and stability of nano emulsions stabilized by nanofibers, but also the great potential of nanofiber particle stabilized Pickering emulsions for the application in food field.

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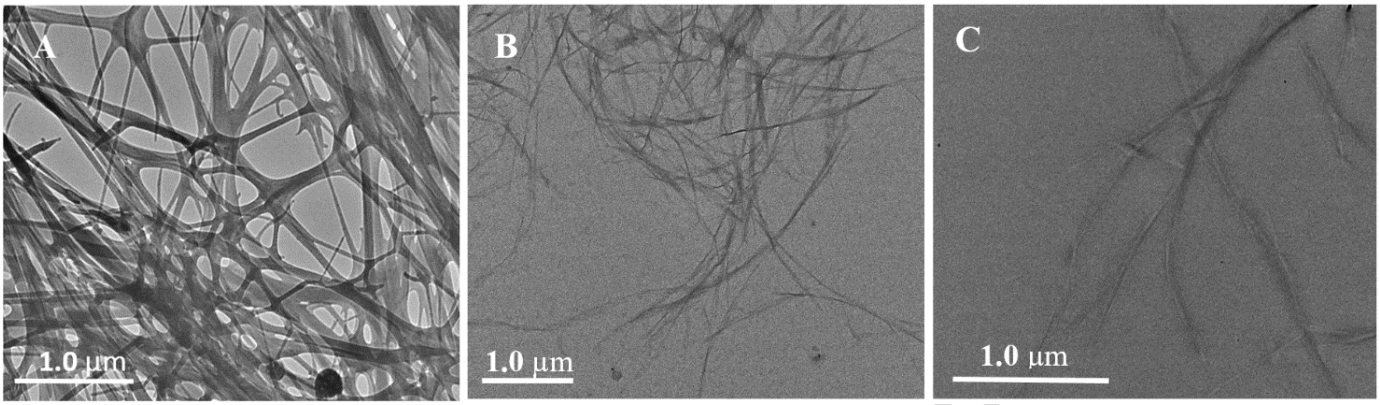


**Fig. 1** TEM images of natural bacterial cellulose (A) and nanofibers from bacterial cellulose by hydrolysis in two magnifications (B and C).

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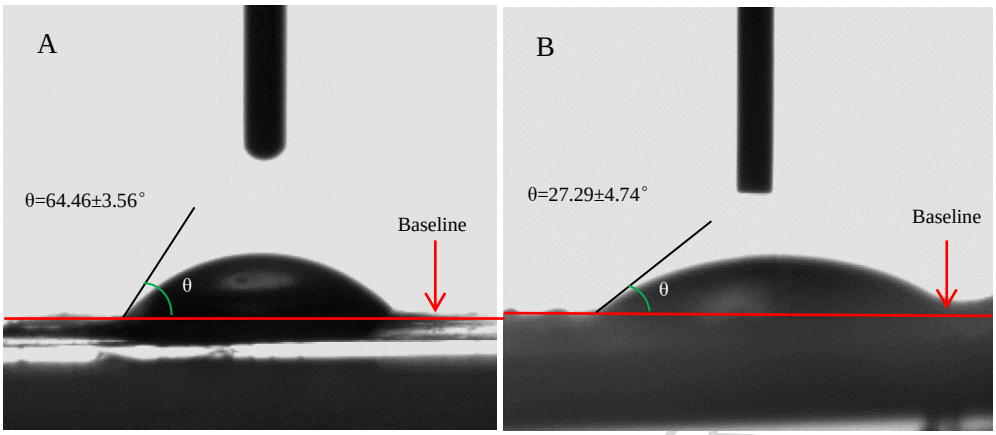


**Fig. 2** Images of contact angles of nanofibers with water (A) and peanut oil (B). θ values (insets) represent the contact angle.

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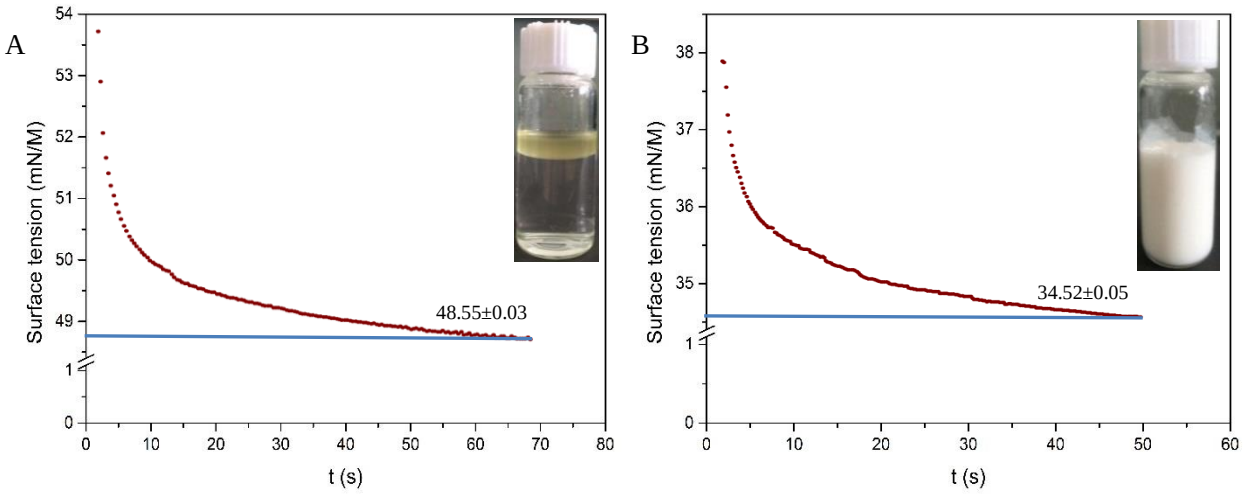


**Fig. 3** Interface tension of oil/water (A) and oil /water emulsions stabilized by nanofibers (B).

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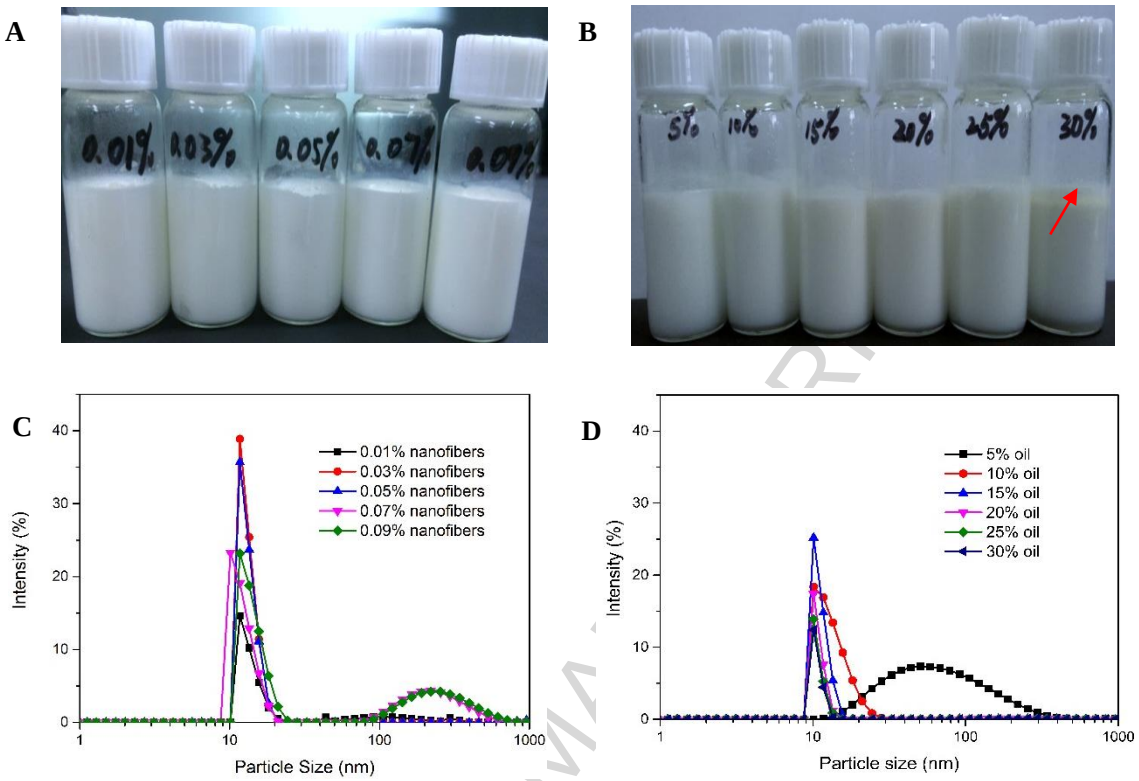


**Fig. 4** Appearance of Pickering emulsions stabilized by different nanofiber concentrations (A) and different oil volumes (B)， and particles size distributions of Pickering emulsions stabilized by different nanofiber concentrations (C) and different oil volumes (D).

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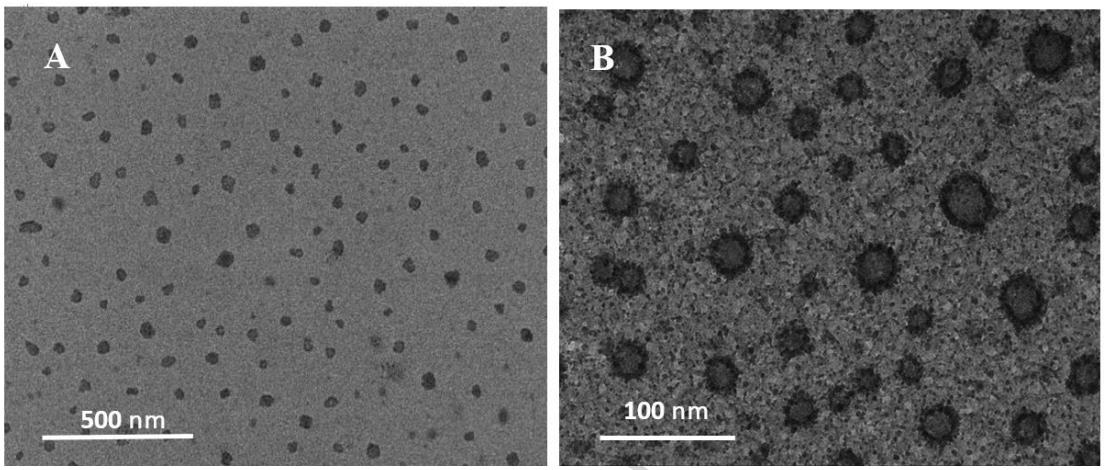


**Fig. 5** TEM images of Pickering emulsions prepared by 0.05% nanofibers and 20% peanut oil at neutral pH in two magnifications (A and B).

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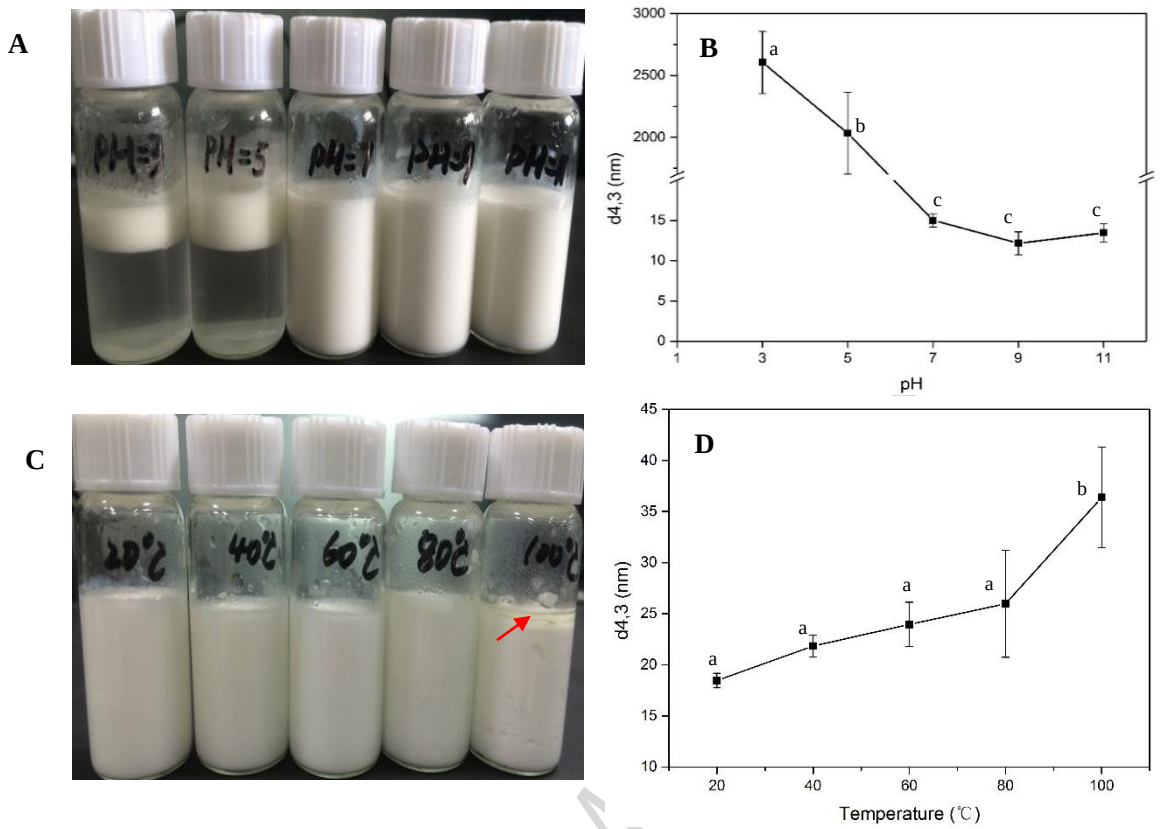


**Fig. 6** Appearance (A) and $d_{4,3}$ (B) of Pickering emulsions prepared at various pH values, appearance (C) and $d_{4,3}$ (D) of Pickering emulsions prepared at various temperatures. Error bars are standard deviations from triplicates. Different letters next to symbols indicate significant differences in the mean ($P < 0.05$).

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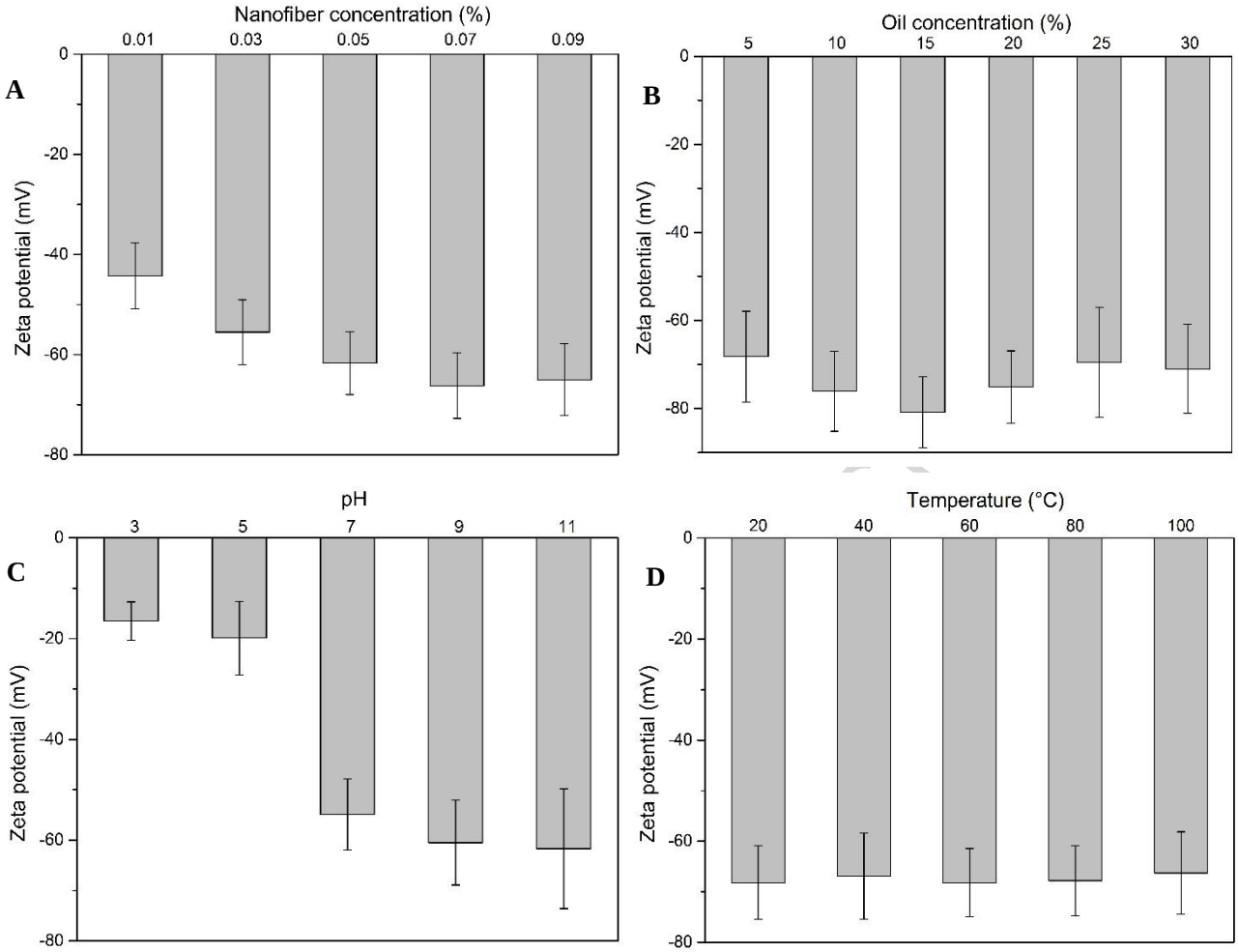


**Fig. 7** Zeta potential of Pickering emulsions prepared at various nanofiber concentrations (A), oil concentrations (B), pH values (C) and temperatures (D).

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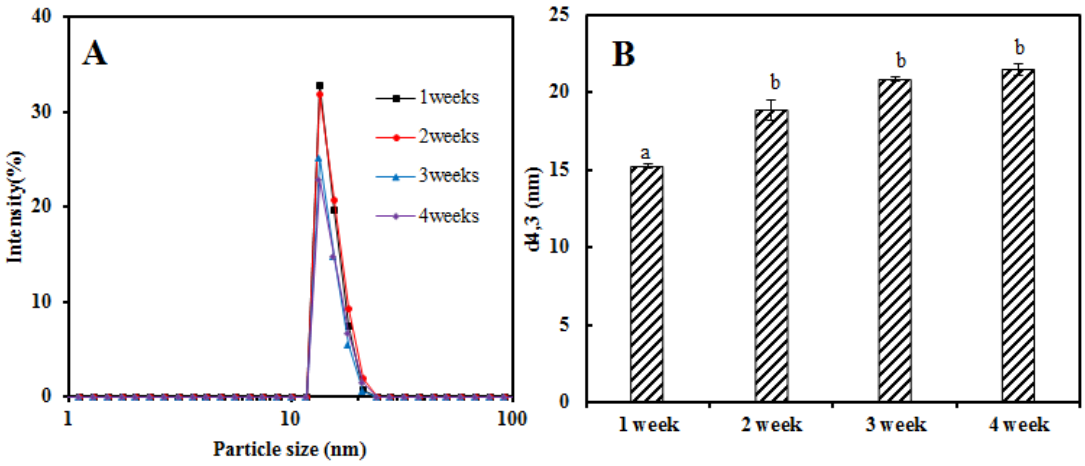


**Fig. 8** Changes of particle size distributions (A) and $d_{4,3}$ (B) of Pickering emulsions prepared with 15% peanut oil (v/v) and 0.05% nanofibers in PBS at pH 7 during storage at room temperature (20 °C) for 4 weeks. Error bars are standard deviations from triplicates. Different letters above bars indicate significant differences in the mean of the same sample ($P < 0.05$).

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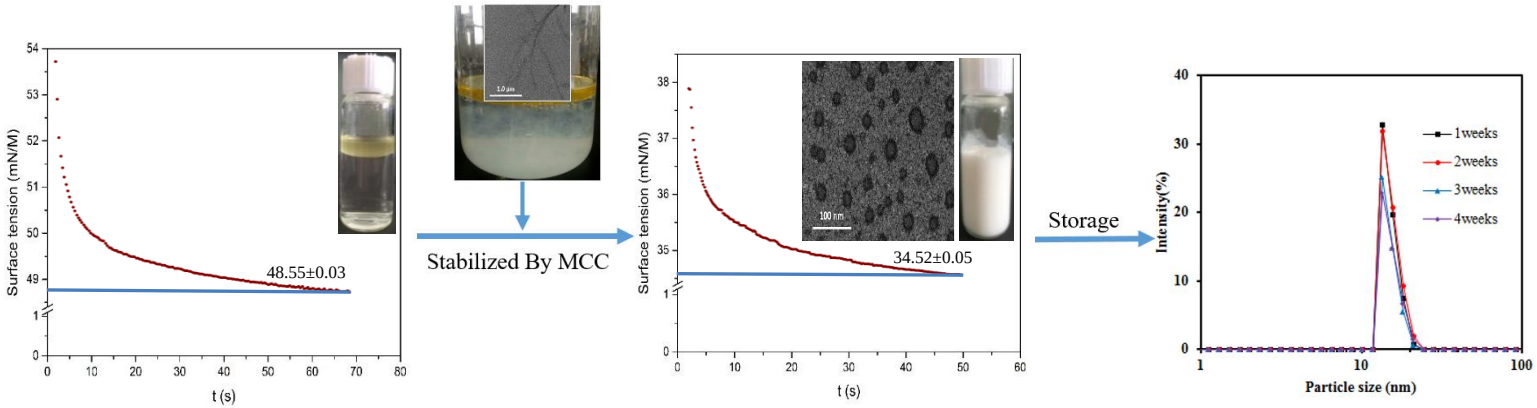
## Graphical abstract

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## Highlights

Nanofibers have significant ability to decrease the surface tension of oil/water droplets.

Only 0.05% nanofibers are able to stabilize oil/water system.

Nanofibers stabilized Pickering emulsions display excellent dispersed properties for 4 weeks.

Nanofibers stabilized emulsions can be used as new potential food-grade Pickering emulsions.

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