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Comparison of emulsifying capacity of two hemicelluloses from moso bamboo in soy oil-in-water emulsions

Yan-Fei Li,[a] Pan-Pan Yue,[a] Xiang Hao,[*a] Jing Bian,[a] Jun-Li Ren,[b] Feng Peng[*a] and Run-Cang Sun[c]

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Oil-in-water food emulsions consisting of natural emulsifiers has been an active field of green scientific inquiry. Here, we extract two types of new hemicellulose-based emulsifiers (H_H and H_L) from holocellulose and dewaxed materials of bamboo (*Phyllostachys pubescens*), as well as compare their emulsifying soy oil ability, respectively. The main content of H_H is arabinoxylan, while the primary composition in H_L is glucan. The emulsifying capacity of these two types of hemicellulose-based emulsifiers are evaluated by droplet size distribution, surface charge and optical microscopy. Since H_L possesses higher lignin and protein residual contents, the resultant emulsion exhibits smaller droplets and higher emulsion stability. In comparison, H_H emulsifier has almost no emulsifying capacity due to the lack of non-polar groups. This study provides insight into the choice of hemicelluloses-based emulsifiers for the formation of stable oil-in-water food emulsions.

1. Introduction

Oil-in-water food emulsions are being developed at a rapid pace because of their many potential applications in food and beverages. In general, they are thermodynamically unstable colloidal systems that need emulsifiers to provide kinetic stability. A large number of emulsifiers have been developed to stabilize food emulsions, including proteins, small colloidal solids, classical monomeric surfactants, and polysaccharides.[1] Given the urgent need for products labelled with clean, demand on the food-grade emulsifier such as polysaccharide grows increasingly. Polysaccharides have properties of thickening and water retention, as well as an emulsification effect on emulsions, due to their hydrophilicity and high molecular weight. The most commonly used polysaccharide emulsifiers in food emulsions are gum arabic, fenugreek, galactomannans, starch, and cellulose derivatives.[2,3] Recent years have witnessed a rapid development on these polysaccharide emulsifiers derived from nature, whereas they are still relatively few compared to synthetic emulsifiers.

In general, there are four factors that affect the emulsifying capacity, including molecular weight, branching degree, the number of lipophilic groups, and protein content. First, the high molecular weight as well as branching degree of polysaccharides plays the role of emulsifying capacity through steric hindrance and charge repulsion.[4,5] Then, polysaccharides from nature generally have lipophilic groups such as acetyl groups or methoxy groups.[6] These lipophilic groups adsorb to the surface of the oil droplets, thereby achieving the emulsification effect. For example, phenolic residues in spruce galactoglucomannans improve the stability of oil-in-water emulsions.[7] In addition, there are physical and chemical interactions between proteins and polysaccharides, thus proteins cannot be completely removed during the extraction and purification of polysaccharides. The emulsifying and stabilizing ability of the polysaccharides are considered to achieve by introducing an amphiphilic residual protein fraction, increasing the steric repulsion and/or the viscosity of the continuous phase. It should be noted that the stability of emulsifiers is also affected by many factors such as heat variability of free proteins and sensitivity to pH. Consequently, even as emulsifiers derived from polysaccharides, there is a need for more research on extracting, characterizing and clarifying the properties of polysaccharide-based emulsifiers from natural sources in a controlled manner.

Among these polysaccharide emulsifiers, hemicelluloses are heteropolysaccharides that they divided into four types, such as xyloglucans, xylans, β-glucans with mixed linkages, and mannans. Unlike starch and cellulose, hemicelluloses derivatives have lower industrial utilization due to low molecular weight and varying chemical composition.[8] Recently, some researchers have tried to exploit the hemicelluloses-based materials to the food emulsion field. For example, Mikkonen

[a]*Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing 100083, China. E-mail: Xianghao@bjfu.edu.cn; fengpeng@bjfu.edu.cn*

[b]*State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China*

[c]*Center for Lignocellulose Science and Engineering, Liaoning Key Laboratory Pulp and Paper Engineering, Dalian Polytechnic University, Dalian 116034, China*

et al.[9] compared the emulsifying and stabilizing capacities of spruce galactoglucomannans (GGM) and its carboxymethyl derivative (CMGGM) with those of known stabilizers, namely, corn fiber gum (CFG) and gum arabic (GA), the latter of which is the gold standard in food emulsions. They demonstrated that GGM and CMGGM were capable of forming emulsions with an average droplet size of approximately 400 nm and stabilizing droplets more effectively than GA. The wood-derived natural emulsifiers GGM and CMGGM exhibited the outstanding emulsification properties, which suggested that hemicelluloses-based material would be potential candidates as emulsifiers for the food industry.

Although some hemicelluloses have been successfully utilized to stabilize oil-in-water food emulsions, most of them are still limited to spruce GGM. Reports on the other types of hemicelluloses to apply in food emulsions are few. In this study, we extract the water-soluble hemicelluloses (H_H and H_L) from holocellulose and dewaxed powder of the bamboo culms, and then examine their emulsifying properties in food emulsions, respectively. The sugar compositions of hemicelluloses are determined by high performance anion exchange chromatography (HPAEC). The lignin and protein residual contents of hemicelluloses are investigated by Klason lignin and the Coomassie brilliant blue G250 (CBBG) methods, respectively. Further, the emulsifying capacity of H_H and H_L for the formation of oil-in-water soy oil emulsions are studied by droplet size distribution, zeta-potential, and morphology analysis. Meanwhile, the role of protein and lignin content in hemicelluloses is described. The results obtained may contribute to choosing appropriate hemicelluloses-based emulsifiers for application in the food and beverage industries.

2. Material and methods

2.1. Materials and chemicals

Moso bamboo (*Phyllostachys pubescens*) was obtained from a local bamboo factory (Sichuan, China). First, it was dried in sunlight and then cut into small pieces (1–3 cm). The chips were dried in sunlight and then grounded to pass a 0.8 mm screen. After being further dried again in a cabinet oven for 16 h at 60 °C. The fats, waxes, and oils of bamboo powder were removed in a Soxhlet apparatus for 6 h with acetone, and the dewaxed bamboo was obtained. All standard chemicals were analytical grade.

2.2. Extraction of H_H and H_L

The water-soluble hemicelluloses (H_L and H_H) were obtained from the dewaxed bamboo and the delignified bamboo (holocellulose), respectively. The dewaxed bamboo was soaked in distilled water at 80 °C for 8 h with solid-to-liquid ratio of 1 : 20 (g/mL). After filtration, the filtrate was evaporated under reduced pressure and freeze-dried to obtain the water-soluble hemicelluloses, labelled as H_L. The holocellulose was obtained by delignification of the dewaxed bamboo (40–60 mesh) with 6 wt% sodium chlorite in acidic solution (pH 3.6–3.8, adjusted by 10 wt% acetic acid) at 75 °C for 2 h. The holocellulose was subsequently extracted with distilled water at 80 °C for 8 h with solid-to-liquid ratio of 1 : 20 (g/mL). After filtration, the filtrate was evaporated under reduced pressure and freeze-dried to obtain water-soluble hemicelluloses, labelled as H_H.

2.3. Chemical characterization

The lignin content of the hemicelluloses was determined by Klason lignin.[10] The protein content of the H_L and H_H was determined by the Coomassie brilliant blue G250 (CBBG).[11] The composition of neutral sugars and uronic acids in the hemicelluloses was determined by high performance anion exchange chromatography (HPAEC). The neutral sugars and uronic acids in the H_L and H_H were liberated by hydrolysis with 10 wt% H_2SO_4 for 2.5 h at 105 °C. After hydrolysis, the sample was diluted 50 times, filtered, and injected into the HPAEC (Dionex ISC 3000, USA) system with amperometric detector, AS50 autosampler and a Carbopac™ PA1 column (4 mm × 250 mm, Dionex).[10]

2.4. Preparation of the emulsions

A series of aqueous emulsifier solutions at different concentrations (0.5 wt%, 1 wt%, 1.5 wt%, 2 wt%, and 2.5 wt%) were prepared by dispersing H_H and H_L in 25 mM citrate buffer (pH 4.5) containing 0.05 w/v potassium sorbate as antimicrobial preservative, and stirred overnight at room temperature (RT). Oil-in-water emulsions were prepared by homogenizing 10 wt% oil phase (soy oil) with 90 wt% aqueous phase. A coarse emulsion was prepared by blending oil and aqueous phases together using a high shear mixer (Ouhor Mechanical Equipment Co., Ltd, Homogenizer, Shanghai) for 4 min at ambient temperature. Fine emulsions were formed by a high pressure homogenizer (Microfluidizer LV1, Microfluidics Inc., Newton, MA). The emulsions were stored at RT.

2.5. Droplet size

The droplet size distribution of the emulsions was determined with static light scattering, using a Mastersizer Hydro 2000 SM (Malvern Instruments Ltd, Worcestershire, UK). The analysis was repeated five times with fresh emulsions and after 1 h, 1 day, 1 week, 2 weeks, and 1 month storage at RT. Refractive indexes of 1.33 for water and 1.47 for dispersed phase were used. Three measurements on each sample were performed. The emulsions were gently turned upside down 10 times before sampling. Emulsions were diluted with buffer solutions of the appropriate pH before analysis to avoid multiple scattering effects. Dilution was necessary to avoid multiple scattering effects, even though this step might disrupt the emulsion system.[12] The droplet diameter of each sample was represented as the volume-weighted mean diameter ($D_{4,3}$) or surface-weighted mean diameter ($D_{3,2}$), which was calculated from the full droplet size distribution.

2.6. Zeta-potential measurement

Zeta-potential was determined using Zetasizer 90 (Malvern Instruments Ltd, USA). All measurements were carried out

according to the method described with slight modifications.[13] For zeta-potential measurement, emulsions were diluted to 0.05 wt% using 25 mM citrate buffer at pH 4.5. Diluted emulsions were injected into a high concentration vial specifically designed for zeta-potential. For measurement of droplet size, the emulsion samples were diluted to approximately 0.005 wt% using 25 mM citrate buffer (pH 4.5). All determinations were conducted in duplicate.

2.7. Optical microscopy

The emulsion morphology was characterized using optical microscopy (AxioScope A1, Carl Zeiss Inc., Oberkochen, Germany). Before tested, the emulsion was mixed gently by turning the container upside down ten times.

3. Results and discussion

3.1. Composition study of H_L and H_H

Since the lignin or protein cannot be completely removed during the extraction and purification of polysaccharides, we study the lignin and protein residual content of H_H and H_L, as can be seen in Fig. 1a. The lignin content of H_L and H_H was determined as 25.50 wt% and 0.67 wt%, respectively. In addition, H_L possessed much higher protein content with 9.53%, while H_H only consisted of 0.90 wt% protein. Taken together, H_L extracted from dewaxed materials of bamboo had much more lignin or protein residual contents.

After confirming the residual contents, we next investigated the sugar composition of these two types of hemicelluloses. As shown in Fig. 1b, the sugar compositions of the H_H were determined as 75.3 mol% xylose, 15.0 mol% arabinose, 7.0 mol% glucose, and 2.7 mol% galactose, which indicated that the main content of hemicelluloses was arabinoxylan. As for H_L, the sugar compositions have been measured as 48.5 mol% glucose, 20.4 mol% galactose, 17.9 mol% arabinose, 10.9 mol% xylose, and 2.3 mol% glucuronic acid, suggesting the main content of glucan. Since the glucan was the main component of H_L, we reasoned that the H_L obtained from dewaxed materials of bamboo contained starch.[14]

FT-IR study was further conducted to confirm the composition of H_H and H_L (Fig. 2). Both of H_H and H_L showed obvious absorption at 3408 cm^{-1} and 2939 cm^{-1}, which were attributed to the O–H stretching vibration of the hydroxyl groups and C–H (CH_2 and CH_3) stretching vibrations in hemicelluloses,[15] respectively. The FT-IR spectra also demonstrated that the hemicelluloses can be obtained from holocellulose without cleaving the acetyl ester groups by the treatment with water, in which the absorption around 1736 cm^{-1} represented the C=O stretching of acetyl groups in the region of the carbonyl stretching vibration. In terms of H_H, the band at 1736 cm^{-1} was ascribed to xylan, whereas it cannot be found in the spectrum of H_L. In addition, the stretching modes around 1240 cm^{-1} and 1249 cm^{-1} were assigned to the C–O linkage in xylan.[16] Evidently, these bands in the spectrum of H_H were much stronger than those of H_L. As for H_L, the characteristic bands at 1598 cm^{-1}, 1509 cm^{-1}, and 1412 cm^{-1} were exploited to confirm the presence of lignin, since they were not overlapped with bands from carbohydrates.[17] In comparison, the absence of band at 1509 cm^{-1} in the H_H spectrum implied that few lignin existed in H_H, which was in accordance with the result of the

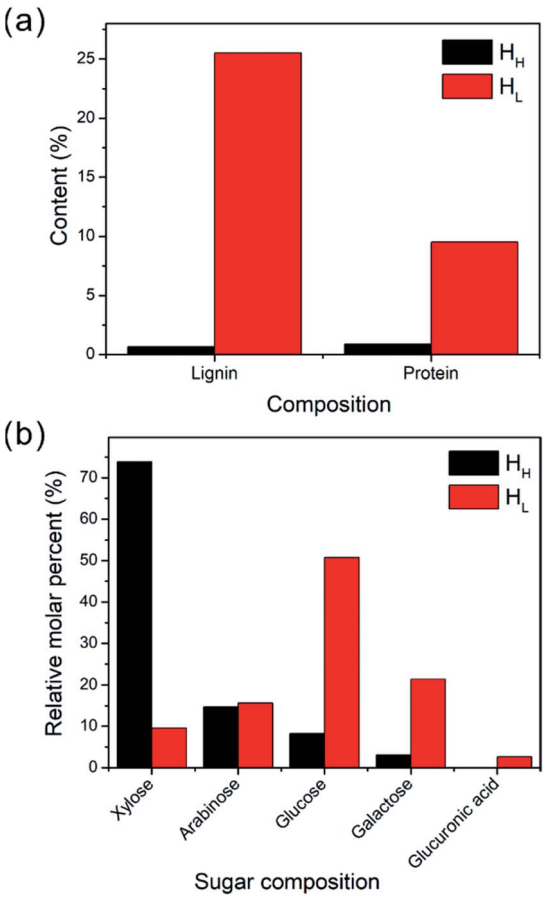

Fig. 1 (a) The lignin and protein composition of H_H and H_L. (b) Sugar composition analysis of H_H and H_L.

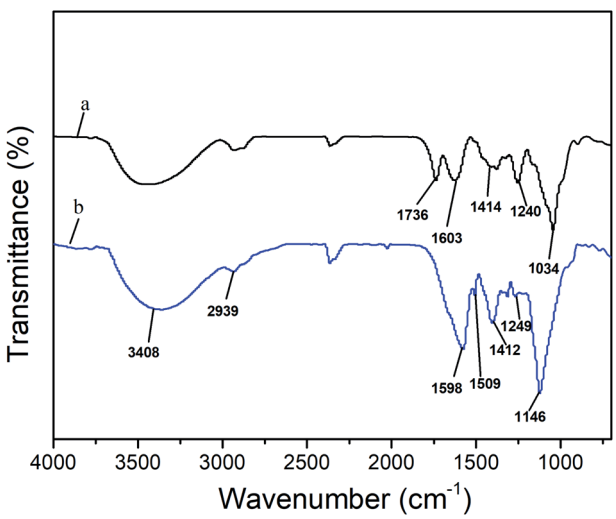

Fig. 2 FT-IR spectra of (a) H_H and (b) H_L, respectively.

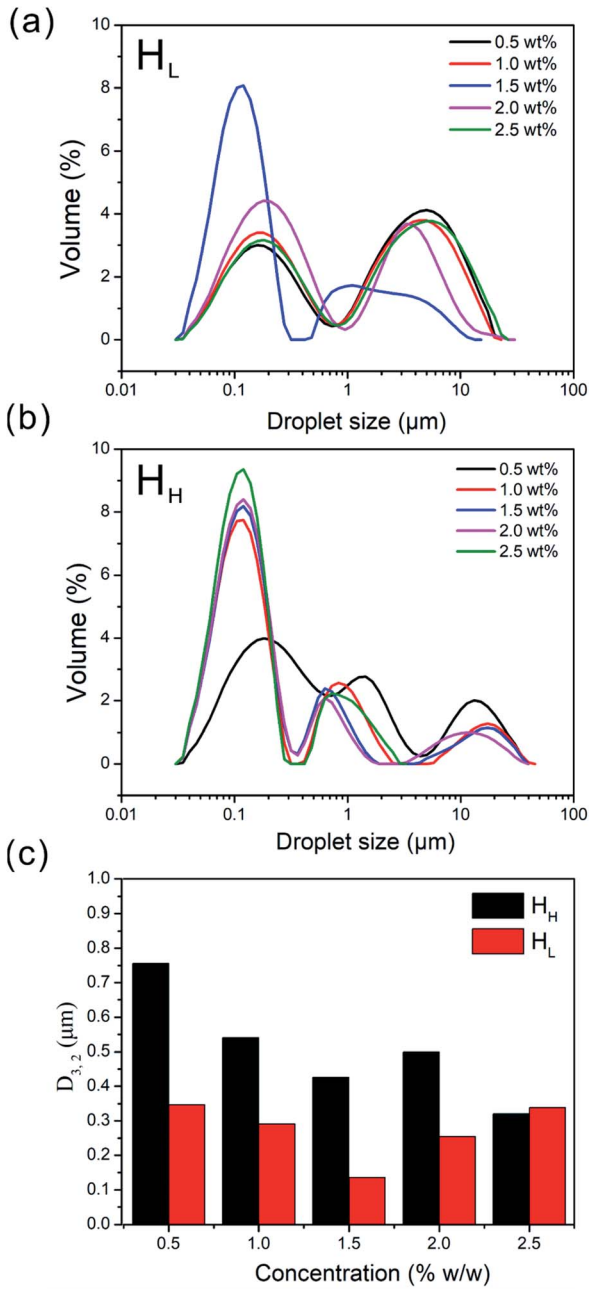

Fig. 3 Droplet size distribution as a function of concentration of (a) H_L and (b) H_H for oil-in-water emulsions. (c) Comparison of the droplet size $D_{3,2}$ for emulsions containing different concentration of H_H and H_L.

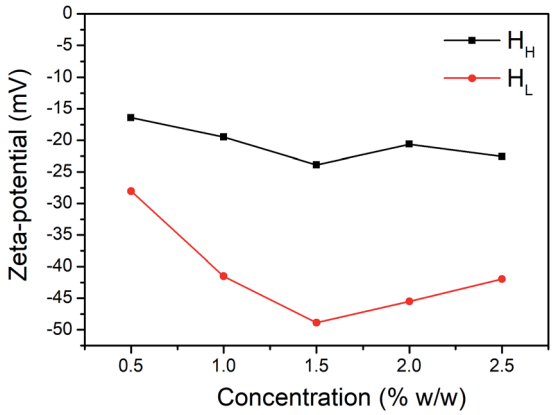

Fig. 4 ζ of H_H-stabilized and H_L-stabilized emulsions as a function of emulsifier concentration.

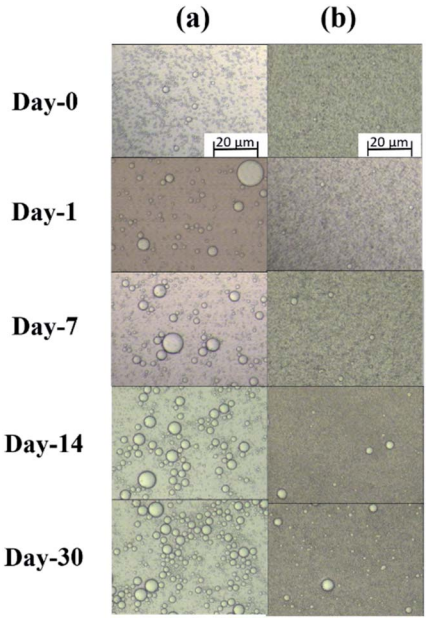

Fig. 5 Optical micrographs of emulsions with 10 wt% soy oil stabilized with 1.5 wt% (a) H_H and (b) H_L at different storage times, respectively. The scale bar is 20 μm, and all images were at the same magnification.

composition of H_H. Evidently, this peak in the spectrum of H_H was stronger than that of H_L. The peaks at 1034 cm^{-1} and 1146 cm^{-1} seem to be due to the C–OH bending mode and C–O stretching in C–O–C glycosidic linkages, respectively.[10]

3.2. Emulsifying capacity evaluation

3.2.1. Influence of hemicelluloses type and concentration on the droplet size distribution. The droplet size distributions of the emulsions formed by H_L and H_H were determined to gain insight into emulsifying capacity. The influences of emulsifier concentration on droplet size distribution for the formation of oil-in-water emulsions were depicted in Fig. 3a–c. All of these emulsions displayed multimodal droplets size distributions. The droplet size distribution of H_L-stabilized emulsions displayed double peak and H_H-stabilized emulsions displayed triple peak in Fig. 3a and b. In terms of H_L-stabilized emulsion, the size distribution was prone to displaying monomodal as the concentration of H_L increased from 0.5 wt% to 1.5 wt%. If the concentration was further increased to 2.5 wt%, the heterogeneity appeared again with the increasing of larger droplets size. A proportion of H_L were not adsorbed on the surface of the oil droplets with the high concentration emulsion system. The increased viscosity produced depletion interaction in the emulsions to bring about the migration of oil droplets through

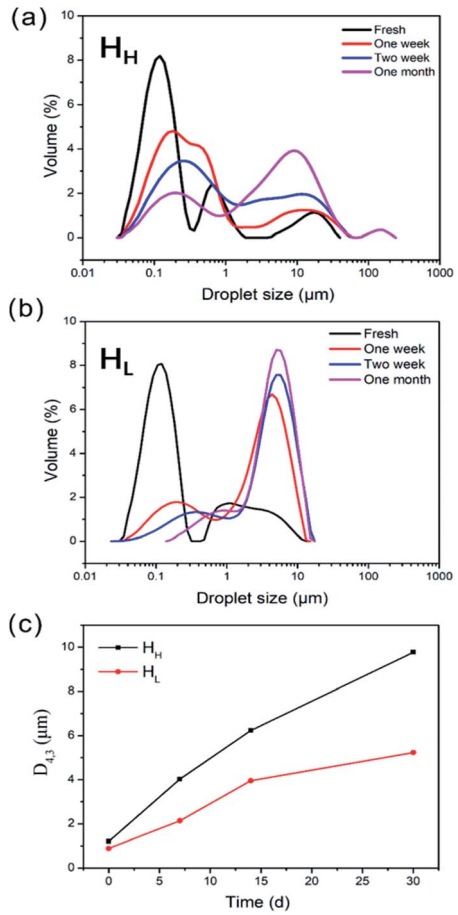

Fig. 6 Effect of time on the droplet size distributions with 10 wt% soy oil stabilized with 1.5 wt% (a) H_H and (b) H_L. (c) Partial size $D_{3,2}$ of emulsions with 10 wt% soy oil stabilized with 1.5 wt% H_H and H_L emulsifiers.

the continuous phase. The droplets approached further to form larger droplets. It was found that 1.5 wt% emulsions can form smaller droplets compared to other concentration. Similar trend were observed by Long *et al.*,[18] in which emulsifier concentration has a great effect on the stability of the emulsions. The droplet surfaces were not effectively covered by emulsifiers, when the emulsifier concentration reached a certain level. In comparison, the H_H-stabilized emulsions exhibited rather inhomogeneity with multimodal droplet size distributions (Fig. 3b). Besides, the diameter distribution of oil droplets can be improved with the increase of the H_H concentration, while the effect of H_H was not comparable to that of H_L.

Emulsions were prepared by mixing soy oil and emulsifiers solutions with different emulsifier concentration at room temperature, and then the surface-weighted mean diameter ($D_{3,2}$) was measured after 1 h (Fig. 3c). As shown in Fig. 3c, the $D_{3,2}$ of H_H-stabilized emulsions was always larger than those of H_L-stabilized emulsions in the concentration ranging from 0.5 wt% to 2.0 wt%. Specifically, the $D_{3,2}$ of the H_L-stabilized emulsions dropped at first and then rose with increasing emulsifier concentration, which suggested that the smallest surface-weighted mean diameter is existed at 1.5 wt% concentration. More emulsifier molecules were adsorbed at the oil–water interface with the increasing of emulsifier concentration, which reduced interfacial free energy in the dispersion system, thereby oil droplets were more difficult to coalesce. However, the increased viscosity caused depletion interaction in a high concentration system and the excess emulsifier in the continuous phase increased the attractive force between the droplets by osmotic effect, whereby the chains of polysaccharides were excluded from the narrow region surrounding the two droplets,[19] so that the droplets size increased. In comparison, the $D_{3,2}$ of the H_H-stabilized emulsions displayed a bigger value than that of H_L. It indicated that the H_H-stabilized emulsions contained much larger droplets than H_L-stabilized emulsions.

3.2.2. ζ-potential study. Zeta-potential (ζ) is an important indicator to characterize the stability of the dispersion system. In general, the greater value of the absolute ζ value, the greater repulsive force between the droplets, resulting in the enhancement the stability of emulsion. The surface potential results showed that ζ of all the emulsions had negative values, wherein the absolute ζ value of the H_L-stabilized emulsion was larger than that of the H_H-stabilized emulsion (Fig. 4). As for H_L-stabilized emulsion, the zeta-potential decreased from −28.06 mV to −48.84 mV with concentration increasing from 0.5 wt% to 1.5 wt%, followed by an increase from −48.84 mV to −41.94 mV with the H_L concentration increasing to 2.5 wt%. Besides, the absolute ζ value of the H_L-stabilized emulsion was larger than that of the H_H-stabilized emulsion, confirming the stability of the H_L-stabilized emulsion. The discrepancy on emulsifying capacity could be attributed to the specific composition of H_L. Before the concentrations of H_L reaching optimum, more lipophilic group of H_L emulsifier would absorb on the droplet interface. Non-absorbed polysaccharides are most likely responsible for promoting droplet flocculation through a depletion mechanism, reducing the chance of mutual coalescence between the oil droplets when the emulsifier was quickly absorbed onto the new oil–water interfaces, thereby making H_L become an effective emulsifier.[20]

3.3. Long-term stability study

3.3.1. Influence of time on morphology. Physical stability of emulsions composed of H_H and H_L was evaluated by optical microscopy. After emulsifying, small micro-size droplets were observed in both of the two fresh emulsions (Fig. 5), wherein the droplets size of H_H-stabilized emulsion was larger than that of H_L-stabilized emulsion. The droplet size became larger with increasing of storage time. After a day, the emulsions were prepared to visualize the existence of droplets smaller than 1 mm that were abundant in the H_L (Fig. 5b) and H_H (Fig. 5a) emulsions. There were a number of larger oil droplets in the H_H-stabilized emulsion, while the larger oil droplets of H_L stabilized emulsion only slightly increased. With the increased storage time, heterogeneous droplets were observed in H_H-stabilized emulsion that oil droplet size differences. The H_L-emulsions also contained some droplets several micrometres in diameter, and the oil phase of the H_H-emulsions was mainly

composed of such large droplets (Fig. 5a). The visual observation and the microscopy of the emulsions indicated that H_L enabled the formation of small oil droplets that made the emulsions more stable against creaming than those containing H_H (Fig. 5).

3.3.2. Influence of time on the droplet size distribution. The droplet size distribution as a function of time for H_L- and H_H-stabilized emulsions were evaluated, as shown in Fig. 6a–c. The H_L-stabilized emulsions showed bimodal droplet size distribution with an increase percentage of large droplet (Fig. 6b). The size distribution of the H_H-emulsions displayed three peaks after one month (Fig. 6a). In addition, nano-size droplets were dominant in the fresh H_L and H_H emulsions, whereas the large micro-droplets became the major part after long time storage. The large droplets developed could also be ascribed to the clusters of droplets, indicating the appearance of flocculates. Despite both emulsions displayed long-term instability, the H_L-stabilized emulsions was relatively stable than H_H-stabilized emulsions.

We then investigated the influence of time on the $D_{4,3}$ of various emulsions, as can be shown in Fig. 6c. After preparation, the $D_{4,3}$ of H_H-stabilized emulsion were approach to H_L-stabilized emulsion with 1.2 μm and 0.9 μm, respectively. However, the $D_{4,3}$ of H_H-stabilized emulsions increased faster than that of H_L with increasing storage time. After one month, the $D_{4,3}$ of H_H-stabilized emulsion was increased to ~10 μm, which was larger than that of H_L-stabilized emulsion with about ~5 μm, confirming the better emulsifying performance of H_L.

4. Conclusions

In summary, we successfully obtain the water-soluble hemicelluloses (H_H and H_L) from holocellulose and dewaxed powder of the bamboo culms, and then compare their emulsifying capacity for oil-in-water food emulsion, respectively. The main content of H_H is arabinoxylan, while the primary composition in H_L is glucan. The H_L type hemicelluloses possess much higher lignin and protein residual content with 25.5 wt% and 9.3 wt%, respectively. In contrast, H_H only consists of 0.67 wt% protein and 0.9 wt% lignin, respectively. The discrepancy of residual content makes these two type hemicelluloses with different emulsifying property. For soy oil-in-water emulsion, the H_L type emulsifier displays much better emulsifying performance than H_H, with relatively uniform size distribution, large ζ absolute value and smaller droplet size. In addition, the H_L-stabilized emulsion shows much long-term stability than H_H-stabilized emulsion, wherein the H_H-stabilized emulsion displays seriously flocculation with the lapse of time. Considering the similar non surface-active inherence of glycosyl units, the emulsifying capacity of H_L is mainly attributed to the non-polar and charge groups brought by the residual content. The findings in this study may provide beneficial information to guide the formation of hemicelluloses-based food emulsion.

Conflicts of interest

There are no conflicts to declare.

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