Characteristics and Rheological Properties of Polysaccharide Nanoparticles from Edible Mushrooms (Flammulina velutipes)

Wenhang Wang, Cong Li, Guanhua Du, Xiuling Zhang, and Hongjie Zhang

Abstract: Nanotechnology has become relevant in the food-related industries, and edible mushrooms can be a potential raw material for providing satisfied edible nanomaterial. In this study, by following 3 different pretreatments (hot water or cold alkali or hot alkali) insoluble polysaccharide nanoparticles were prepared from Flammulina velutipes by wet milling and high pressure homogenization and their properties were investigated. The resultant nanoparticles were characterized by SEM, GC-MS (for its main compositions), FTIR, XRD, and TG. The 1 wt% nanoparticle dispersions presented non-Newtonian, shear-thinning fluids with the viscosity in an increasing order for the hot water < cold alkali < hot alkali. Moreover, the dynamical rheological results showed differences of storage ($G^\prime$) and loss ($G^\prime\prime$) moduli of these particle dispersions. It was concluded that the Flammulina velutipes-derived polysaccharides nanoparticles have great potential applications in the food industry, for example, as emulsifiers, reinforcement agents, and bioactive carriers.

Keywords: edible mushroom, physic-chemical properties, polysaccharide nanoparticles, rheological characteristics

Practical Application: The Flammulina velutipes-derived polysaccharides nanoparticles have great potential applications in the food industry, for example, as emulsifiers, reinforcement agents and bioactive carriers

Introduction

Nanotechnology is a popular subject in the research community (Sun and others 2014; Tang and others 2014), and it has also become relevant to the food industry, with the potential to increase agricultural productivity, food security, and economic impact for industries (Handford and others 2014). As a multidisciplinary field, nanotechnology in the food industry may include the overall manufacturing, processing, safety, packaging, transportation, storage, and delivery (Chau and others 2007) and the following 3 applications are of particular interest: agri-food including nano-formulated agrochemicals, animal feeds, and nano-biosensors; food processing involved in nano-sized ingredients, additives, nutritional supplements, and functional foods (Truong and others 2015); and incorporation into some packaging or coating that come into contact with food (Rossi and others 2014).

To enhance the application of nanotechnology in the food industry, finding new nanoscale edible materials becomes necessary. In the literature, some nano food grade materials (fiber or particles) have been reported, including starch (González and Villanueva 2011), fat (Truong and others 2015), chitosan (Chantarapatporn and others 2014), protein (Foegeding 2006), and cellulose (Olsson and others 2011).

Edible mushrooms may be a suitable raw material for producing nanomaterial for food applications due to their large amount of insoluble fibrous polysaccharides, mild hardness, and popularity. Edible mushrooms refer to a wide range of fungus for human consumption, which mainly belong to the basidiomycota family, such as Flammulina velutipes, Lentinus edodes, Pleurotus ostreatus, Pleurotus tuber-regium, Tricholoma matsutake, Auricularia auricular, and so on. Up to now, there are about 200 kinds of edible mushrooms in the world, with differences in chemical composition, nutritional value, and a variety of physiological functions (Kalal 2013). The main composition in edible mushrooms is polysaccharide, in which the water insoluble polysaccharide is predominant, and its content is more than 90% (Wong and others 2003; Wong and Cheung 2005). So, edible mushrooms, as a potential function of dietary fiber source (Cheung 2013), can be made into drinking and special dietary fiber food products, which have already attracted much attention (Wong and others 2005; Wong and Cheung 2009). Dietary fibers are defined as edible plants or carbohydrates, which are resistant to digestion and absorption in the human small intestine, and have beneficial physiological effects, such as laxation and blood glucose attenuation (Wong and Cheung 2009). Compared to other conventional dietary fibers derived from cereals, fruits, legumes, and vegetables, edible mushroom-based insoluble dietary fibers are underutilized, providing a great opportunity for further development, in particular, new functional food nanomaterials.

Flammulina velutipes is one of the most popular edible mushrooms owing to its high nutritional values and attractive taste, with production and consumption ranked at 4th place among edible mushrooms in the world (Cai and others 2013). F velutipes has various beneficial health effects for humans (Cheung 2013), exhibiting good antioxidant, anti-inflammatory, immunomodulatory, antitumor, and cholesterol-lowering activities (Kang and others 2014). Aside from interesting bioactive compounds in the fungus, the abundant fibers play an important role in the biological benefits. In this study, we aimed to prepare new nano
polysaccharide particles from *F. velutipes*, by employing 3 different pretreatments: hot water or cold alkali or hot alkali, followed by a series of wet milling, high pressure homogenization. The resultant nanoparticles were further analyzed based on microscopy, chemical composition, high conformation, and thermo stability. Furthermore, their rheological characteristics were investigated with steady shear viscosity and dynamic frequency sweep test, providing some basic information for its potential food applications.

**Materials and Methods**

**Materials and reagents**

The fruit bodies of *F. velutipes* (89.37% moisture, 2.72% protein, and 5.45% carbohydrate, w/w) were obtained from Raoyang mushroom Co. Ltd (Hebei, China). Trifluoroacetic acid, sodium borohydride, hydrochloric acid, methyl alcohol, ethyl alcohol, pyridine, acetic anhydride, and sodium hydroxide were supplied by Jiangtian Chemical Co. Ltd (Tianjin, China) without further purification for use.

**Preparation of polysaccharide nanoparticles**

Preparation of polysaccharide nanoparticles from the fruit bodies of *F. velutipes*. Briefly, after heating at 100 °C for 5 min to decrease the enzymatic activity, the fruit bodies were respectively subjected to different treatments of hot water or hot alkali or cold alkali with the solid-to-liquid ratio 1:3, followed by washing with a great amount of Mill-Q water until pH became neutral. Then, the 3 pretreated samples were ground by using PFI-MILL (PTI P40110.E00, Austria) at 20 MPa 2 times and then were further treated by ultra-high pressure homogenizer (NS1001L, Niro Soavi, Italy) at 100 MPa for 5 times. The resultant nanoparticles were centrifuged (TGL-16B, Anting Scientific Instruments Factory, Shanghai, China) at 12000 g for 12 min and then collected, which were diluted to 1 wt% nanoparticle dispersion. The hot water, hot alkali, and cold alkali pretreated *F. velutipes* nanoparticles (FNP) were respectively named as FNPhw, FNPha, and FNPCA.

**Micromorphology of FNP**

FNP dispersion of 20 μL was added into 40 mL acetone, which was diluted to a concentration of 0.002% and was dispersed by using ultrasonic cleaners (SB25-120, Scientz Biotechnology Co. Ltd, Ningbo, Zhejiang) for 30 min at room temperature. The morphology of the prepared samples was performed on a Scanning Electron Microscope (SEM) (JSM-IT300LV, Hitachi, Japan) at an accelerating voltage of 10 kV.

**Yield and chemical composition of FNP**

The ash content was determined by ashing the 3 FNP at 525 °C for 5 h in a temperature-controlled muffle furnace (SX2-4-10, Xinhuan Instrument Co. Ltd., Zhengzhou, Henan, China) as described previously (Lee 1990). The protein content was analyzed by Coomassie brilliant blue protein method (Sedmak and Grossberg 1977). The yield was cacurated according to the following equation:

\[ \text{Yield (\%)} = \frac{A_1}{A_0} \times 100\% \]  \hspace{1cm} (1)

where \(A_1\) is the residue quality after pretreatment, and \(A_0\) is the total weight of *F. velutipes*.

The monosaccharide composition of FNPs was measured as described in the literature (Yang and others 2012). Briefly, FNPs of 15 mg was hydrolyzed with 4 mL, 2 mol/L of trifluoroacetic acid at 110 °C for 4 h. The hydrolyte was reacted with hydroxylamine hydrochloride and acetylated with 0.5 mL of acetic anhydride. The acetylated samples were subjected to gas chromatograph, coupled with a mass spectrometer (4000M, Virian Technology, USA), according to a previous method (Santos–Neves and others 2008).

**Results and Discussion**

**Yield and chemical composition of FNPs**

With a series of wetting, grinding, and high pressure homogenizations, FNPs from the pretreated fruit bodies of edible mushrooms (*F. velutipes*) were prepared with different yields: 49.2% for hot water pretreatment, 42.2% for cold alkali pretreatment, and 39.4% for hot alkali pretreatment (Table 1). It was found from Table 1 that the monosaccharide composition of 3 FNP samples was mainly glucosamine and glucose with small amounts of mannose, rhamnose, and galactose, indicating that FNPs are residue rich in sugars, which will be polymerized into glucan and chitin. The above results agreed with those reported in the literature (Chen and Cheung 2014). Moreover, it can be derived from...
Table 1–Chemical composition and yield of 3 FNPs with different pretreatments.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Glucose</th>
<th>Glucosamine</th>
<th>Mannose</th>
<th>Rhamnose</th>
<th>Galactose</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FNPhw</td>
<td>3.50 ± 0.03a</td>
<td>0.33 ± 0.05b</td>
<td>39.80</td>
<td>51.75</td>
<td>2.21</td>
<td>1.28</td>
<td>4.97</td>
<td>49.20</td>
</tr>
<tr>
<td>FNPhc</td>
<td>2.28 ± 0.09e</td>
<td>0.27 ± 0.02b</td>
<td>29.91</td>
<td>63.79</td>
<td>2.16</td>
<td>1.05</td>
<td>3.10</td>
<td>42.22</td>
</tr>
<tr>
<td>FNPha</td>
<td>2.49 ± 0.07b</td>
<td>0.22 ± 0.02b</td>
<td>39.75</td>
<td>53.37</td>
<td>2.25</td>
<td>1.03</td>
<td>3.60</td>
<td>39.43</td>
</tr>
</tbody>
</table>

Data of ash and protein represent mean values ± standard deviation (n = 3). Different letters within the same column indicate statistically significant differences (P < 0.05).

FNPhw, hot water pretreatment; FNPha, hot alkali pretreatment; FNPhc, cold alkali pretreatment.

Table 1 that high purified FNPs would be obtained due to the obvious reducing of ash content and protein. As shown in Table 1, the ash contents of the 3 samples were 3.5%, 2.28%, and 2.49%, respectively, and the contents of the 2 alkali-treated samples were lower than that of the hot water-treated sample, which implied that the minerals and impurities which are present in the mushroom were removed more effectively under alkaline pretreatment condition. Additionally, the 3 nanoparticle samples had much lower protein content, compared with the raw material of F. velutipes, suggesting that all pretreatments could solubilize the protein present in the mushroom, resulting in a less protein content.

Compared with the FNPhw and FNPha, FNPhc contained more glucosamine while less glucose and galactose, presumably due to the structure variation of these polysaccharide particles resulting from the difference of pretreatments. This result was mainly due to the following reason: the mushroom cell wall could easily be destroyed by alkali pretreatment, which would make more polysaccharide dissociate out of cell wall, and lead to more chitin present in FNPhc. However, hot alkali will cause serious damage to chitinous structure, reflected by the low content of glucosamine that was present. The similar result also had been found that glucose, glucosamine mannose, galactose, and rhamnose were the main components of the fungal cell wall polysaccharides in the fruit bodies of F. tuber-regium, P. rhinocerus, and W. cocos, with the varying ratios of them as being subjected to different treatments of hot water and alkali (Ka-Hing and Cheung 2005).

Micromorphology

Figure 1 showed the SEM images of FNPs derived from F. velutipes. It was found that these particles presented a uniform but irregular subglobular structure and the diameter of the majority particles nearly ranged from several tens to hundreds of nanometers. These results supported the conclusion that polysaccharide nanoparticles can be prepared based on pretreatment of hot water or hot alkali or cold alkali, followed by mechanical homogenization, which can provide functional nanomaterials for food application.

Furthermore, the average size of the 3 samples was slightly different depending on the pretreatment methods. And the FNPhw sample had the biggest size (100 to 300 nm), whereas the size of FNPha was the smallest (50 to 100 nm), which was due to the fact that the hot alkali pretreatment condition offered the best condition to dissolve the soluble polysaccharides and other biopolymers presented in the F. velutipes, thus facilitating the formation of nanoparticles in the subsequent mechanical homogenization step.

Infrared spectra

Figure 2 displayed the FTIR spectra of 3 FNPs extracted from F. velutipes with different pretreatment methods, and the peak position together with the spectra intensity were listed in Table 2. The absorption peak at 890 cm⁻¹ was ascribed to the β configuration of glucan (Fariña and others 2009). Furthermore, the characteristic peak at 1555 cm⁻¹ referring to amide group (–CO-NH-C) was observed in FNPhc, which indicates the presence of a large amount of chitin and is consistent with that of a glucan-chitin complex (Kozarski and others 2011; Chen and Cheung 2014). The changes in characteristic peaks at 1555 cm⁻¹ were consistent with the monosaccharide analysis of these particles in the present study, further confirming that a relative high content of chitin existed in FNPhc while a relatively large amount of glucan in FNPhw and FNPha. The change of O-H stretching at 3400 cm⁻¹ was caused by the different moisture content in FNPs, presumably due to their structure variation. Additionally, the absorption peak at 1150 cm⁻¹ belonging to one of the polysaccharide characteristic peaks suggests that the 3 FNPs contain pyranose monomers in their structures (Zhang and others 2013).

X-ray diffraction

As shown in Figure 3(A), one broad peak at 20° was observed for the 3 FNPs, which were generally similar, suggesting the low crystallinity and amorphous nature of FNPs, which were associated to the crystalline contribution of the chitin (Robles and others 2016). Importantly, compared with the other 2 samples (FNPhw and FNPha), the FNPhc sample had a unique peak at 31.7°, presumably owing to a high glucan presence (Anjugam and others 2016). Apparently crystallinity increased for FNPhc, which was due to the elimination of amorphous polysaccharides during the pretreatments.

Thermal properties

All samples exhibited 2 classical heat endothermic processes (Figure 3B), which belong to the dissociation of hydrogen binding and the thermal depolymerization of polysaccharide particles, respectively. These particles showed different peak temperatures: FNPha (88.86 °C, 262.29 °C), FNPhc (87.54 °C, 291.19 °C), and FNPhw (80.74 °C, 293.76 °C). The difference in the 1st thermal transition’s peak temperatures might be attributed to the different moisture contents in the 3 polysaccharides (Kong and others 2015). After alkali treatment, more hydrophilic groups, such as carboxyl were exposed, which can bind more water molecules, leading to a higher water content in the samples. While for the latter thermal transition, the differences in peak temperatures might be ascribed to the differences in particle size and microstructure of these FNPs as shown in Figure 1, closely associated with the pretreatment-dependent changes in the cell wall of F. velutipes.

From Thermogravimetric Analysis (TG) and Derivative Thermogravimetric Analysis (DTG) curves (Figure 3C and D), there was a weight loss of around 80 °C and 200 to 300 °C respectively, which coincides with the DSC analysis. The initial weight loss was due to the moisture evaporation owing to the intermolecular dehydration and decomposition of the particles (Albu and others 2014). The latter was the major degradation of the polysaccharide structure (Iqbal and others 2013).
Rheological property

The rheological property of the 3 FNPs was studied and the results were shown in Figure 4. A rapid decrease in viscosity was observed in all samples with increasing the shear rate, indicating that all dispersions behave as non-Newtonian (pseudo plastic), shear-thinning fluids (Volk and others 2015). This is attributed to the disruption of physical forces, such as hydrogen binding, Van der Waals’ force and hydrophobic interaction, as a result of shearing force. Similar shear-thinning behavior was also observed in cellulose nanocrystals suspension isolated from cotton (Qiao and others 2015).

Furthermore, the viscosity of FNPs’ dispersions was in the order of FNPhw < FNPca < FNPha. The differences should be mainly attributed to the variance of the size of these particles. With decreasing size, particles interaction increased, resulting in high viscosity (Boylu and others 2004; Viamajala and others 2009). Additionally, the functional groups and related structure of these particles may partly influence their viscosity.

Moreover, the dynamic viscoelastic properties of FNPs dispersions were shown in Figure 5. First, FNPhw dispersion had smaller elastic modulus and viscous modulus than the FNPha and FNPca dispersion (Figure 5A), presumably associated with larger particle
Table 2–Peak position and intensity of FTIR spectra of 3 FNP samples derived from *F. velutipes* with different pretreatments.

<table>
<thead>
<tr>
<th>Peak wave number cm⁻¹ (intensity)</th>
<th>Assignment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>891(S) 893(W) D</td>
<td>Glucan stretch</td>
<td>(Fariña and others 2009)</td>
</tr>
<tr>
<td>1154(S) 1150(S) 1142(W)</td>
<td>C-O stretch</td>
<td>(Zhang and others 2013)</td>
</tr>
<tr>
<td>D 1543(S) 1555(W) 1551(W)</td>
<td>NH bend couple with CN stretch</td>
<td>(Chen and Cheung 2014)</td>
</tr>
<tr>
<td>1640(S) 1636(W) 1651(W)</td>
<td>C=O stretch</td>
<td>(Zhang and others 2013)</td>
</tr>
<tr>
<td>2852(W) 2853(W) 2852(S)</td>
<td>CH₂ symmetrical stretch</td>
<td>(Synytsya and others 2009)</td>
</tr>
<tr>
<td>2923(W) 2923(W) 2923(S)</td>
<td>C-H stretch</td>
<td>(Zhao and others 2010)</td>
</tr>
<tr>
<td>3416(S) 3405(S) 3385(S)</td>
<td>-OH stretch</td>
<td>(Yang and others 2006)</td>
</tr>
</tbody>
</table>

S, strong; W, weak; D, diminished.

size. Second, *G’* became constant at a strain of 1% to 2% in all FNP dispersions, but *G''* was nearly constant (Figure 5A), therefore a constant strain of 2% within the linear viscoelastic region was used in the following experiment.

Figure 5(B) shows the curves of frequency sweeping of FNP, in which these particles presented increasing storage (*G’*) and loss (*G''*) moduli as frequency increasing due to their alignment and disentanglement. The extent of increasing in *G’* and *G''* of FNP dispersions varied upon pretreatment, which could be attributed to the properties of these particles, such as size, composition. FNP dispersions presented a behavior of *G’* > *G''* at lower frequencies (1 to 6 Hz), indicating a gel-like behavior, but opposite in higher
Characteristics and rheological properties...

Figure 4—Viscosity of 3 water dispersions of FNPs from *F. velutipes* with different pretreatments of hot water, hot alkali, and cold alkali.

Figure 5—(A) Strain scan (1 wt%, 1 Hz, 25 °C) and (B) dynamic oscillatory rheology (1 wt%, 25 °C, 2% strain) of 3 dispersions of FNPs from *F. velutipes* with different pretreatments of hot water, hot alkali, and cold alkali.

frequency (68 to 100 Hz). Among these FNPs, FNPhw showed a more pronounced change in viscoelasticity as frequency increased, which might be attributed to the rearrangement of these larger particles to form a more regular and compact network. As noted from Figure 5(B), a crossover of $G'$ and $G''$ values was obtained at frequencies of 31, 46, and 31 Hz, for FNPhw, FNPha, and FNPca dispersions, respectively. The crossover frequency provided a good indication of the viscoelastic behavior of the material (Brito and others 2005), and the lower the crossover value, the larger the elastic contribution (Ramachandran and others 1999). Therefore, it could be derived that FNPhw and FNPca dispersions indicates a more elastic behavior than that of FNPca dispersion. This difference might be due to the different interactions between FNPs related with particle size.

Conclusions
Following various pretreatments, polysaccharide nanoparticles with size of 50 to 300 nm were successfully prepared from the fruit bodies of edible mushrooms *F. velutipes* by a series of wetting, grinding, and high pressure homogenization. These particles are mainly composed of glucose and glucosamine, with small amounts of mannose, rhamnose, and galactose, and negligible amount of protein, with observable differences in chemical composition and molecular conformation closely associated with pretreatment method. The tougher treatment, such as alkali, can reduce particle size and decrease thermal stability, which should be ascribed to effective dissociation of the fungal cell fragments it caused. Although generally showing non-Newtonian, shear-thinning flowability, these particles presented some different rheological properties in terms of storage moduli ($G'$) and loss moduli ($G''$), partly due to differences in particle size. The potential impact of these pretreatments on surface activity, absorption capacity, and charge density of these particles is still needed to explore for completely illustrating the complex link among pretreatment, physicochemical properties, and food-related processing features. In summary, given the aforementioned exclusive characteristics,
the *F. velutipes*-derived polysaccharides nanoparticles provide the potential for food applications, for example, as emulsifiers, reinforcement agents, and bioactive carriers.

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