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Effect of high-pressure homogenisation-modified bacterial cellulose on rice starch retrogradation

[1,2]Xie, X. H., [1,2]Zhu, H. S., [1,2]*Zhang, B. B., [1,2]Xu, C., [1,2]Zhang, B. and [1,2]Qi, L.

[1]*College of Food Science and Technology, Henan Agricultural University, Zhengzhou, 450002, Henan Province, China*
[2]*Key Laboratory of Staple Grain Processing, Ministry of Agriculture, Zhengzhou, 450002, Henan Province, China*

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Abstract

Delaying rice starch (RS) retrogradation can improve the quality parameters of rice-based starchy foods during storage. Modification of insoluble dietary fibre has always been used in the starchy food industry. Compared with vegetal insoluble dietary fibre, bacterial cellulose (BC) has many advantages such as high purity, smaller particle size, and elevated water absorption capacity. In the present work, BC was modified by high-pressure homogenisation (MBC) with different pressure levels (0, 50, 80, 120, and 160 MPa) to investigate the effect of MBC on RS retrogradation. Results showed that high-pressure homogenisation could decrease the particle size of BC. MBC addition to RS decreased paste breakdown and setback, thus suggesting that MBC might be a good candidate for increasing the stability of RS paste, and inhibiting its short-term retrogradation. The thermal properties and X-ray diffraction patterns of RS indicated that supplementing MBC could decrease the gelatinised enthalpy and relative crystallinity of RS paste during storage. Results also indicated that MBC could provide an opportunity to restrain RS retrogradation, and might be suitable for designing fibre-enriched products.

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Introduction

Rice serves as a cereal crop, and provides 20% of the global calories consumed by humans. Rice starch (RS) is the major component of milled rice, and accounts for about 80% of the dry weight of rice grain. RS is unique with a creamy, spreadable, and smooth texture. These properties have increased the demand for RS in various foods such as noodle, rice vermicelli, and sweet dumpling (Yoenyongbuddhagal and Noomhorm, 2002; Malahayati *et al.*, 2015; Lin *et al.*, 2021). However, gelatinised-rice-based starchy foods easily age, thus leading to brittle RS with poor water stability (Yamaguchi *et al.*, 2019). Therefore, various ingredients and additives are being studied to slow RS retrogradation.

Dietary fibre is used as an ingredient to inhibit short- and long-term retrogradation of starchy food (Lai *et al.*, 2011; Tang *et al.*, 2013). It can be divided into soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). Although the predominant fibre fraction in the by-products of many crops is IDF, SDF rather than IDF is always used in foods (Aravind *et al.*, 2012), because the solubility of SDF in water effectively locks available water in starch molecules (Lai *et al.*, 2011; Tang *et al.*, 2013). In comparison, IDF swells and entraps water in its porous structure (Mudgil, 2017). Therefore, adding IDF causes the unsuitable technological properties of the supplemented products (Robin *et al.*, 2012). Efforts have been made to improve the physicochemical properties of IDF. For example, the smaller particle size of IDF has a stronger restraining effect on retrogradation and maintains the stability of RS paste (Liu *et al.*, 2016). Therefore, more and more IDF have been used in food production. Bacterial cellulose (BC) is a unique type of IDF, and produced by aerobic bacteria (Shi *et al.*, 2014; Corral *et al.*, 2017). Unlike IDF, which requires harsh chemical treatment to be isolated from plants, BC has high purity, and is lignin- and hemicellulose-free (Iqbal *et al.*, 2014; Gallegos *et al.*, 2016). BC can be produced in films, particles, and spheres. It can enhance food stability, improve the gel strength of tofu, show a superior water-holding capacity, and improve the network structure of surimi (Lin *et al.*, 2011; Shi *et al.*, 2014; Gomes *et al.*, 2018). Moreover, BC is nanosized with a high surface area,

*Corresponding author.
Email: zhangbobohqwzb@163.com

and can form a 3D network structure with high porosity, which can increase moisture retention and delay the retrogradation of wheat starch in bread (Corral *et al.*, 2017). Additionally, high-pressure homogenisation (HPM) is regarded as one of the most effective means to increase the specific surface area of BC, and enhance its water adsorption capacity (Li *et al*., 2019; Bevilacqua *et al*., 2019). Hence, the effect of HPM on BC in inhibiting RS retrogradation is essential to be investigated.

Although researchers have reported that the properties of BC are multifunctional, the effect of the particle size of BC on RS quality remains unclear. Additionally, although HPM has been reported to change the particle size of IDF (Liu *et al*., 2016), the feasibility of HPM on BC needs to be verified. Therefore, the present work aimed to explore the effect of HPM on the particle size of BC, and investigate the potential use of HPM-modified BC (MBC) as an ingredient of rice-based starchy food.

Materials and methods

Materials

RS was obtained from Wuhu Haoyikuai Co. Ltd. (Anhui, China). BC produced by *Gluconacetobacter xylinus* was provided by Hainan Guangyu Biotechnology Co. Ltd. (Hainan, China).

Effect of HPM on particle size of BC

BC was treated by HPM as described previously with some modification (Wang and Wang, 2011). BC and deionised water (1:35, m/v) were mixed and then homogenised at 0, 50, 80, 120, and 160 MPa at 25°C for five cycles with an AH-PILOT 2015 homogeniser (ATS Engineering Inc., Suzhou, China). Then, all MBCs were spray freeze-dried by a SP-3000 lab spray freeze dryer (Sunyi Tech. Co. Ltd., Shanghai, China).

The particle size of the MBC suspension was analysed by a LS-13 laser diffractometer (Beckman Coulter Commercial Enterprise Co. Ltd., China), with water as the solvent. Samples were diluted 1:10 (v/v) with solvent, and mixed for 3 min, then applied to the instrument. All measurements were carried out at room temperature in triplicate.

Effect of MBC on pasting properties of RS

The pasting properties of RS+MBC samples were determined by RVA-4500 Rapid Visco-Analyser (Perten, Australia) (Liu *et al*., 2016; Xiao *et al*., 2017). Briefly, MBC (0.15 g, dry weight basis) was dissolved in distilled water with continuous magnetic stirring for 2 h. RS (2.85 g, dry weight basis) was dispersed in MBC solutions by stirring for 30 min at room temperature to avoid lump formation. Then, water was added to obtain a total weight of 28 g. The above samples were equilibrated at 50°C for 1 min, heated to 95°C at a rate of 12°C/min, and then held at 95°C for 2.5 min. The sample was subsequently cooled to 50°C at a rate of 12°C/min and maintained at 50°C for 2 min. The paddle speed was set at 960 rpm for 10 s, and then maintained at 160 rpm during measurement to disperse the sample.

Effect of MBC on gelatinisation and retrogradation properties of RS

The gelatinisation properties of RS+MBC samples were determined by differential scanning calorimetry (DSC; DSC-214, Netzsch Co. Ltd., Germany) following a previous method (Yan *et al*., 2021). MBC (0.15 g, dry weight basis) was dissolved in distilled water (12 mL) with continuous magnetic stirring for 2 h at 25°C. RS (2.85 g, dry weight basis) was dispersed in MBC solutions by stirring for another 1 h. Then, 8 mg of the slurry was transferred into an aluminium DSC pan. All samples were equilibrated at 4°C for 24 h. Then, gelatinisation was measured from 25 to 100°C at the rate of 10°C/min, followed by cooling to 25°C at the same rate. Then, gelatinisation curves were obtained. Gelatinisation enthalpy (ΔH_g) was determined from the curves.

The retrogradation properties of the samples were determined by DSC-214 and X-ray diffraction (XRD) as follows: the gelatinised samples were stored at 4°C for 1, 7, 14, 21, and 28 days to assay the effects of MBC on RS retrogradation. Then, 8 mg of each sample was transferred into an aluminium DSC pan. The procedure was performed from 25 to 100°C at the rate of 10°C/min, followed by cooling to 25°C at the same rate. Then, retrogradation enthalpy (ΔH_R) was determined from the curves. The retrogradation percentage (R%) was calculated by dividing the ΔH_R of the second heating run at 28 days by ΔH_g in the first heating run (Temsiripong *et al*., 2005; Yan *et al*., 2021). Analyses were performed in triplicate.

The recrystallisation of the gelatinised RS determined by XRD reflected the RS retrogradation. Briefly, RS and MBC (5 wt.% on starch basis) were dispersed in deionised water at 25°C by magnetic stirring for 30 min, and gelatinised at 95 °C in a water bath for 20 min. All the samples were cooled to room

temperature, stored at 4°C for 28 d, and spray freeze-dried by the SP-3000 lab spray freeze dryer. The freeze-dried samples were milled to pass through a 150-mesh sieve. XRD was carried out using an X'Pert Pro X-ray diffractometer (PANalytical, the Netherlands), which was equipped with a copper tube operating at 40 kV and 30 mA Cu-Kα radiation. Diffractograms were obtained by scanning from 4° (2θ) to 40° (2θ) at a rate of 2°/min. MDI Jade 5.0 was used to analyse the relative crystallinity (RC).

Effect of MBC on microstructure of RS

Scanning electron microscopy (SEM; Quanta-250, FEI Ltd., USA) was used to assay the effect of MBC on the microstructure of RS. Briefly, RS and MBC (5 wt.% on starch basis) were dispersed in deionised water at 25°C by magnetic stirring for 30 min, and gelatinised at 95°C in a water bath for 20 min. All the samples were cooled to room temperature, stored at 4°C for 28 days, and freeze-dried. Subsequently, the samples were cut into 3 mm thickness, stuck on a specimen holder, and coated with gold-palladium using a sputter coater. The microstructures of the samples were observed at 250 resolutions. The porosity of the RS gels was assayed by MATLAB (Matrix Laboratory 2016a software).

Statistical analysis

Results were expressed as mean ± standard deviation (SD) of triplicate analyses for each sample. One-way ANOVA and Tukey's test were performed to establish the significance of differences among the mean values at the 0.05 significance level. The statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, USA).

Results and discussion

Effect of HPM on particle size of BC

The effect of HPM on the average particle size of BC was studied. As depicted in Figure 1, the particle size distribution of BC decreased considerably with increasing HPM pressure. For example, the particle size of BC after HPM treatment at 80 MPa (MBC_{80}, 14.36 μm) was remarkably smaller than that of native BC (MBC_0, 25.42 μm). The BC modified by HPM_{120} had a smaller particle size (MBC_{120}, 7.94 μm). Therefore, the effect of HPM on the particle size of BC was pressure-dependent. Clarke *et al*. (2010) reported that HPM coupled with pressure and heat tends to shift granules from loose into compact structure with more cavities on the surface. However, when compared with HPM treatment at 120 MPa, the particle size of BC with HPM treatment at 160 MPa was enhanced to 8.15 μm (MBC_{160}). In other studies, HPM-modified maize amylase at a treatment pressure higher than 120 MPa had more aggregated structure than that at 120 MPa (Tu *et al*., 2013). The phenomenon observed in the present work might have resulted from the enhancement of the electrostatic attraction of granules when the particle size was smaller than a certain value, as tiny particles are easy to reaggregate (Visser, 1989).

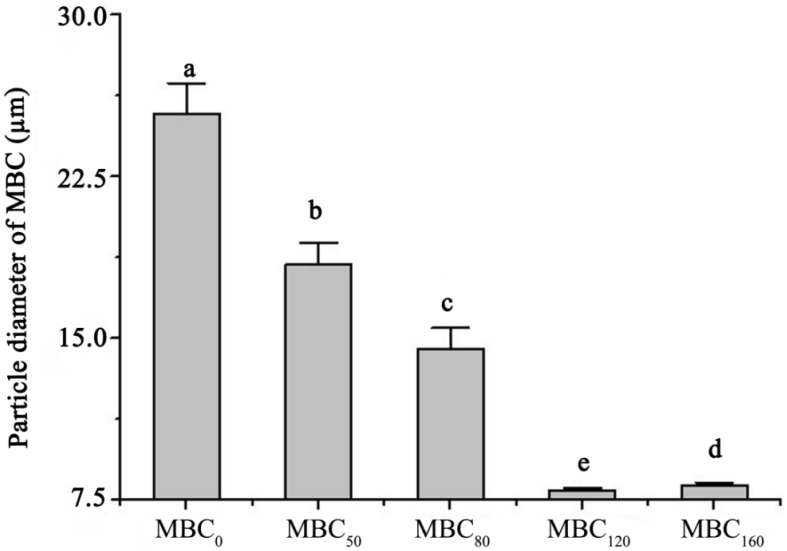

Figure 1. Effect of HPM on the particle size of BC. MBC_0, MBC_{50}, MBC_{80}, MBC_{120}, and MBC_{160} are bacterial celluloses modified by HPM with 0, 50, 80, 120, and 160 MPa, respectively. Different lowercase letters indicate significant differences ($p < 0.05$).

Effect of MBC on pasting properties of RS

The pasting properties of RS in the presence of MBCs with different particle sizes are summarised in Table 1. When compared with native RS, the peak viscosity of RS with MBC addition considerably decreased, thus indicating that MBC supplementation decreased the movement rate between swollen RS particles. In agreement with our results, HPM-modified soybean IDF decreased the viscosity of RS because of its opened structures with a higher degree of porosity, and more exposed surface area and water-binding sites than native IDF (Lin *et al*., 2014; Liu *et al*., 2016), which led to the decrease in the movement rate between swollen RS particles (Hongsprabhas *et al*., 2007; Wu *et al*., 2009). Moreover, amylopectin determines starch viscosity. Polysaccharides could inhibit the hydration of amylopectin, thus leading to a decrease in starch viscosity (Tester and Sommerville, 2003). Therefore, the amylopectin of RS might not completely form viscous substances without sufficient water, owing to the moisture absorption of MBC with high water-binding sites or high degree of porosity.

The breakdown value indicates the stability of starch during the heating and stirring processes. A lower breakdown value reflects a greater granule integrity. Our results showed that the breakdown value of RS paste decreased from 477.33 to 242.30 mPa·s with the addition of MBC from 50 to 160 MPa. This indicated that the stability of the RS paste was considerably increased by MBC addition, and the effect was more remarkable for the MBC with a smaller particle size.

The setback value of RS significantly ($p < 0.05$) decreased from 593.67 to 230.31 mPa·s with the addition of MBC with particle sizes from 25.42 to 7.94 μm. The setback value indicates the recrystallisation degree and internal rearrangement of amylose molecules during the cooling process of starch paste (Luo *et al*., 2017). This indicated the reductive effect of MBC on the setback value of RS, which might have been related to the inhibition of amylose rearrangement caused by the hydrated layer formed by MBC around the starch granules.

Table 1. Effects of MBC on the pasting parameters of RS.

Sample	Peak viscosity (mPa·s)	Trough viscosity (mPa·s)	Final viscosity (mPa·s)	Breakdown (mPa·s)	Setback (mPa·s)
RS	2792.67 ± 28.02^{a}	2089.33 ± 29.77^{b}	2733.67 ± 31.37^{a}	538.67 ± 14.74^{b}	644.33 ± 4.16^{a}
RS+MBC_0	2628.00 ± 29.51^{b}	2162.33 ± 5.51^{a}	2762.00 ± 7.94^{a}	592.76 ± 8.32^{a}	593.67 ± 8.33^{b}
RS+MBC_{50}	2583.33 ± 60.04^{c}	2106.67 ± 38.73^{a}	2654.67 ± 6.03^{b}	477.33 ± 6.03^{c}	548.00 ± 8.66^{c}
RS+MBC_{80}	2250.00 ± 29.14^{d}	1813.67 ± 27.15^{c}	2261.33 ± 3.51^{c}	437.32 ± 3.51^{d}	448.33 ± 4.93^{d}
RS+MBC_{120}	2085.33 ± 22.12^{e}	1895.00 ± 21.17^{c}	2125.33 ± 22.12^{d}	190.21 ± 5.03^{f}	230.31 ± 5.03^{f}
RS+MBC_{160}	1987.33 ± 3.21^{f}	1745.00 ± 18.74^{d}	2014.23 ± 29.54^{e}	242.30 ± 19.40^{e}	269.30 ± 13.28^{e}

RS: rice starch; MBC_0, MBC_{50}, MBC_{80}, MBC_{120}, and MBC_{160}: bacterial celluloses modified by HPM with 0, 50, 80, 120, and 160 MPa, respectively. Different lowercase superscripts in the same column indicate significant differences ($p < 0.05$).

Effect of MBC on gelatinisation and retrogradation properties of RS

The effects of MBC on the gelatinisation and retrogradation properties of RS are shown in Table 2. The ΔH_g of RS remarkably decreased from 12.13 to 9.32 J/g when the particle size of MBC decreased from 25.42 to 7.94 μm. This suggested that the addition of a smaller MBC induced a lower ΔH_g. Consistent with our results, the addition of IDF with smaller particle sizes and more degree of porosity induced a lower ΔH_g in RS (Liu *et al*., 2016). ΔH_R is associated with the degree of starch retrogradation (Hoover and Senanayake, 1996).

The effects of MBC on the ΔH_R and R% of RS paste are shown in Table 2. The results showed that the ΔH_R of RS paste drastically increased from 2.78 to 7.66 J/g as the storage time increased from 1 to 28 days. This trend was also found in corn starch during storage (Li *et al*., 2016). Moreover, the addition of a smaller MBC resulted in a RS paste with lower ΔH_R and R%, thus suggesting that the effect of MBC on RS retrogradation was dependent on particle size.

Table 2. Effects of MBC on the gelatinisation enthalpy, retrogradation enthalpy, and retrogradation ratios of RS.

Sample	ΔH_g (J/g dry starch)	ΔH_R (J/g dry starch)					R%
		1 Day	7 Day	14 Day	21 Day	28 Day	
RS	12.94 ± 0.14^a	2.78 ± 0.12^a	4.67 ± 0.27^a	6.28 ± 0.25^a	7.09 ± 0.23^a	7.66 ± 0.16^a	59.20
RS+MBC_0	12.13 ± 0.12^b	2.34 ± 0.02^b	4.20 ± 0.06^b	5.10 ± 0.10^b	5.52 ± 0.01^b	6.37 ± 0.15^a	52.51
RS+MBC_{50}	11.65 ± 0.17^c	2.21 ± 0.03^b	3.74 ± 0.01^c	3.94 ± 0.0.5^c	4.67 ± 0.07^c	5.18± 0.02^c	44.46
RS+MBC_{80}	10.74 ± 0.05^d	1.91 ± 0.05^c	3.33 ± 0.03^d	3.61 ± 0.02^d	4.39 ± 0.01^d	4.43 ± 0.18^d	41.25
RS+MBC_{120}	9.32 ± 0.16^f	1.09 ± 0.02^d	2.51 ± 0.01^e	2.77 ± 0.02^e	3.35 ± 0.02^e	3.55 ± 0.06^e	38.09
RS+MBC_{160}	9.89 ± 0.17^e	1.21 ± 0.03^d	2.53 ± 0.57^e	2.82 ± 0.02^e	3.38 ± 0.02^e	3.85 ± 0.05^e	38.93

RS: rice starch; MBC_0, MBC_{50}, MBC_{80}, MBC_{120}, and MBC_{160}: bacterial celluloses modified by HPM with 0, 50, 80, 120, and 160 MPa, respectively. Different lowercase superscripts in the same column indicate significant differences ($p < 0.05$).

The RC values of the samples stored for 28 days at 4°C, assayed by XRD, are illustrated in Figure 2. RC could reflect the proportion of crystals in starch. The crystals were formed by the cross-linking of amylopectin, which occurs in the long-term retrogradation of samples (Luo *et al*., 2017). The RC of RS+MBC_0 (11.76%) was higher than those of RS+MBC_{50} (9.96%) and RS+MBC_{120} (8.53%). Therefore, the effect of MBC on the RC of RS was pressure-dependent, thus indicating that MBC had a retarding effect on amylopectin recrystallisation and long-term RS retrogradation. Previous study showed that the addition of polysaccharides decreased the formation of hydrogen bonds between wheat starch molecules and the delayed retrogradation of wheat starch because of its water-binding capacity (Funami *et al*., 2008). In the present work, the MBC with a smaller particle size might possess a higher superficial fraction and more water-binding sites, and inhibit the interaction between RS molecules. MBC might also bind to starch via hydrogen bonds, thus resulting in the decrease in hydrogen bond formation between RS molecules and the delay of RS retrogradation.

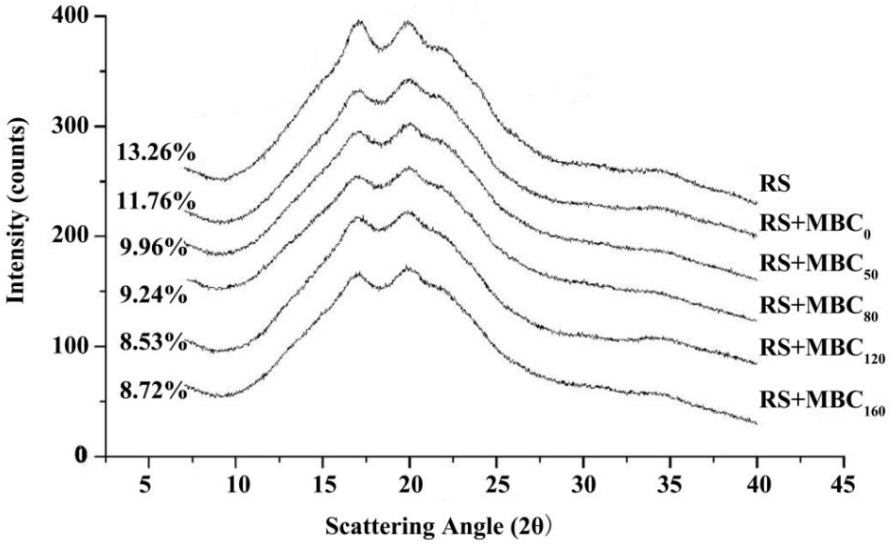

Figure 2. Effect of MBC on the XRD patterns of RS. RS: rice starch; MBC_0, MBC_{50}, MBC_{80}, MBC_{120}, and MBC_{160} are bacterial celluloses modified by HPM with 0, 50, 80, 120, and 160 MPa, respectively.

Effect of MBC on microstructure of RS gel

The morphology of RS gel was observed by SEM. Results are shown in Figure 3. All samples developed a honeycomb-like network structure with different pore sizes. The RS gel without MBC showed a loose structure with large pores. The addition of MBC decreased the pores in the honeycombed structure of the RS gel in a particle size-dependent manner. The structural characteristics of the sample were responsible for the strength of the RS gel network, thus further verifying the delayed effect of MBC on RS retrogradation.

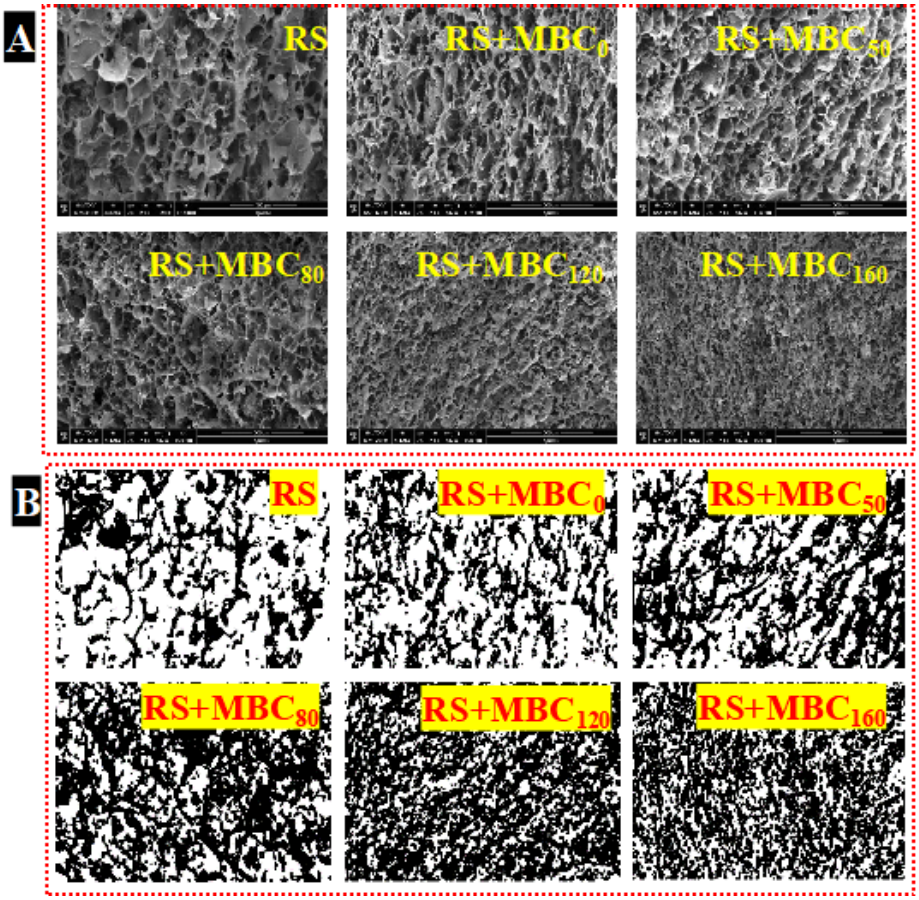

Figure 3. Effect of MBC on the morphological structures of RS [**(A)**: Original images; **(B)**: Porosity images]. RS: rice starch; MBC_0, MBC_{50}, MBC_{80}, MBC_{120}, and MBC_{160} are bacterial celluloses modified by HPM with 0, 50, 80, 120, and 160 MPa, respectively. Different images in the same column are the same sample.

Conclusion

The present work herein verified the effects of MBC particle size on the retrogradation behaviours of RS. Results demonstrated that HPM could be used to modify MBC to obtain MBCs with different particle sizes. MBC reduced the peak viscosity, breakdown value, and setback value of RS, thus suggesting that it inhibited the short-term retrogradation of RS paste. Moreover, MBC considerably reduced the retrogradation enthalpy and relative crystallinity of RS paste. MBC also participated in the formation of the network structure of RS gel. Therefore, MBC had a remarkable inhibiting effect on the long-term retrogradation of RS gels. Overall, our findings indicated that MBC could be applicable to inhibit RS retrogradation and prolong the shelf life of rice-based starchy foods.

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