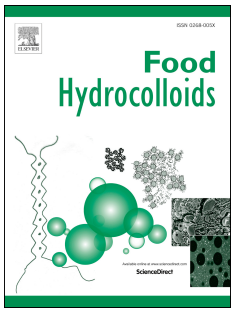

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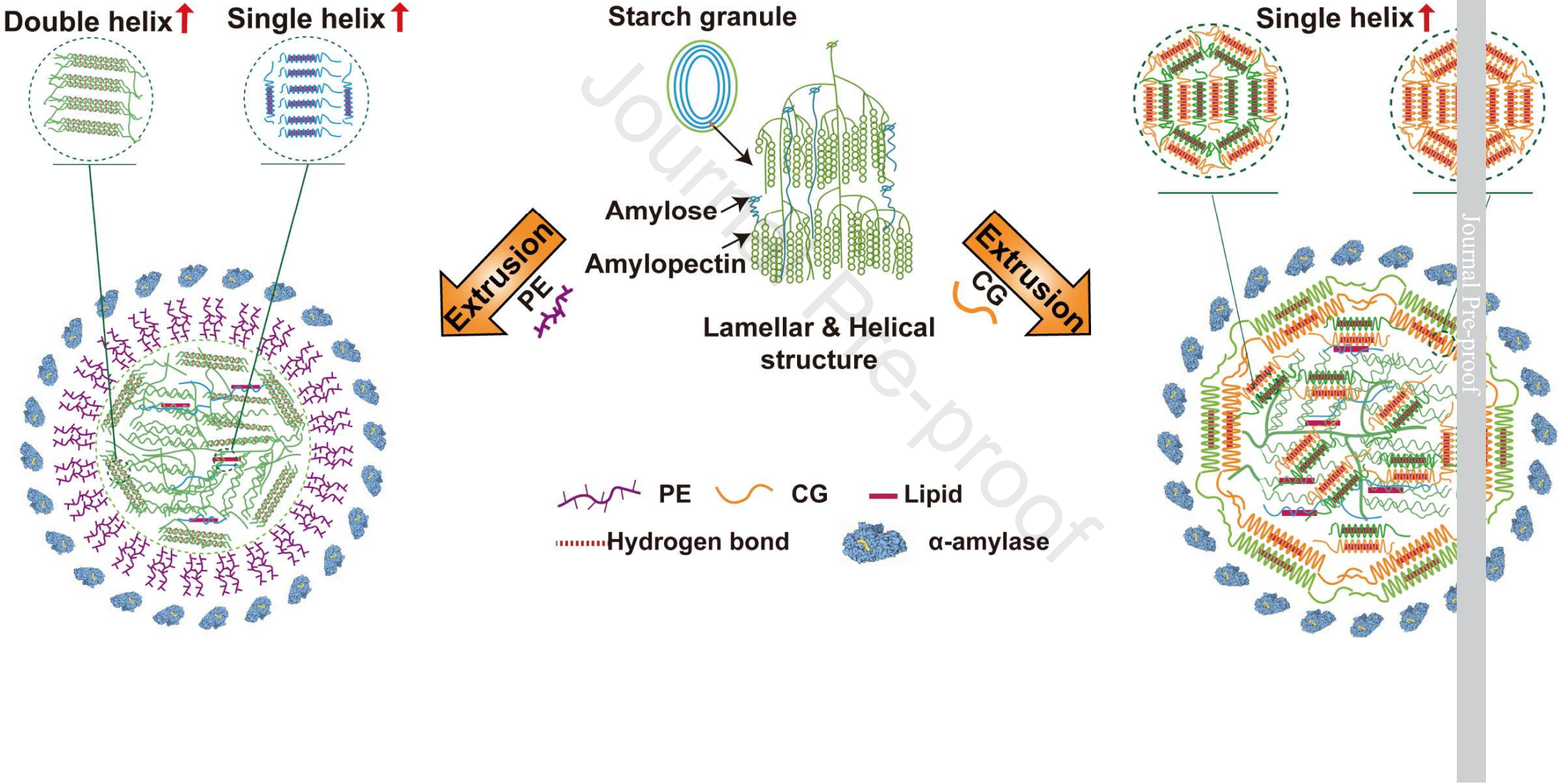

Different effects of pectin and κ-carrageenan on the multiscale structures and *in vitro* digestibility of extruded rice starch

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Abstract

This study compared the different effects of pectin (PE) and κ-carrageenan (CG) on the multilevel structures (*i.e.* granule, crystallites, helices, fractals, and short-range order) and *in vitro* digestibility of extruded rice starch (MERS). The addition of either PE or CG altered the multiscale structures, increased the resistant component (RC) content, and reduced predicted glycemic index (pGI), with CG being more effective especially at a low content. CG led to the growth of molecular aggregates (α), single helices, and V-type crystals (X_V). PE resulted in higher contents of double helices, A+B-type crystals (X_{A+B}) and enhanced the short-range order ($R_{1045/1022}$) and particle compactness. Pearson correlation analysis indicates that, for MERS/PE, the slowly-digestible component (SDC) and RC contents were influenced by different factors in the sequence of total crystallinity (X_{Total}) > X_{A+B} > X_V > single-helix content > α > $R_{1045/1022}$ > double-helix content. For MERS/CG, the RC content was affected by X_{Total} > single-helix content > X_V.

Keywords: Pectin; Carrageenan; Extrusion; Rice starch; Multiscale structure; *In vitro* digestibility

1 Introduction

Rice is the typical staple food and most critical cereal crop in Asian (Marshall & Wadsworth, 1994). Starch is the main ingredient of rice, which plays an essential role in human nutrition (Czech, Pastuszak, & Kusior, 2014). Unfortunately, gelatinized rice starch has more than 95% rapidly digestible starch (RDS) (Zheng et al., 2018), which can cause negative physiological effects such as high glycemic response.

The enzymatic digestion behavior of cooked rice may be changed with addition of some non-starch polysaccharides (NSPs) (Chung, Liu, & Lim, 2007). The underlying mechanism for this change could be different depending on NSP structure, with the most typical explanation being an increased viscosity of intestinal contents (Brennan, Suter, Luethi, Matiamerino, & Qvortrup, 2008). Furthermore, NSPs have been reported to reduce starch digestion by forming a “barrier” on the starch granule surface against amylase (Dartois, Singh, Kaur, & Singh, 2010; Tomoko Sasaki & Kohyama, 2012; Sasaki, Sotome, & Okadome, 2015). Moreover, NSPs may also inhibit α-amylase in a direct non-competitive matter, which restrain starch digestion and, hence, inhibit postprandial glycemia (Slaughter, Ellis, Jackson, & Butterworth, 2002).

Pectin (PE) is an important NSP. It is composed of the galacturonic acid main chain and neutral sugar side chains, which contains approximately 65% homogalacturonan (HG), 20−35% rhamnogalacturonan I (RGI), 10% rhamnogalacturonan II (RGII), and small amounts of xylogalacturonan (XG) (Guo et al., 2016; Naqash, Masoodi, Rather, Wani, & Gani, 2017). Based on the degree of methoxylation (DM), PE can be divided into high-methoxyl PE (HMP) and low-methoxyl PE (LMP). In addition, κ-Carrageenan (CG) is an NSP consisting of sulfureted or

53 non-sulfated galactose and 3,6-anhydrogalactose that are alternately connected by α-1,3 glycosidic
54 bonds and β-1,4 bonds, while the 1,3-linked D-galactose unit C_4 carries a sulfate group (Ueda, Itoh,
55 Matsuzaki, Ochiai, & Imamura, 2001; Viebke, Borgström, Carlsson, Piculell, & Williams, 1998).
56 How the structural difference between PE (branched chain) and CG (linear chain) can lead to
57 different effects on the digestion of rice starch is interesting but has rarely been reported.

58 In most studies, simple physical blending of starch with NSPs with abundant water was used to
59 investigate the effects of NSPs on the digestibility of starch (Anynda, Kelvin Kim Tha, Allan Keith,
60 & Lara, 2019; Gularte & Cristina, 2011; Tomoko Sasaki et al., 2012; Tester & Sommerville, 2003).
61 Besides, the digestibility of starch–NSP composites subjected to physical modification such as
62 heat-moisture treatment and extrusion has also been reported (Adamu, 2001; Chen, Xiong, & Gao,
63 2017). Our previous work (He et al., 2020) has shown that the complexation between rice starch and
64 guar gum could be assisted by thermomechanical treatment. However, the enzymatic digestibility of
65 rice starch–NSP composites thermomechanically processed with limited moisture content have still
66 been limited.

67 Extrusion has promising application prospects in the food manufacturing industry owing to its
68 benefits of simple operation, environmental friendliness, and high safety (Tran, Hendriks, & van der
69 Poel, 2008). Many studies have proved that the paste properties (*e.g.* rheology) of starch will change
70 greatly during the extrusion process (Bhattacharya & Hanna, 1987; Cai & Diosady, 1993; Chuang &
71 Yeh, 2004; Fishman, Coffin, Konstance, & Onwulata, 2000). Nonetheless, extrusion is normally a
72 complex process, during which the precise control of starch structural changes is challenging due to
73 the difficulties in manipulating the residence time and thermomechanical history of each small

74 portion of the processed material. To overcome these difficulties, a miniature twin-screw extruder
75 providing simplified, well-controlled, and more uniform processing conditions was employed in this
76 current study to investigate the functions of PE or CG on the digestibility of extruded rice starch
77 (MERS) in a more accurate way. Based on this, we have established the relationship between the
78 multiscale structures (*i.e.* granule, crystallites, helices, fractals, and short-range order) and the *in*
79 *vitro* digestibility of the thermomechanically-processed composites.

80 2 Materials and Methods

81 2.1 Materials

82 Rice starch (GABIOSTA-F) was supplied by Jinnong Biotechnology Co., Ltd (China); Pectin
83 from Aladdin Biochemical Technology Co., Ltd. (China); κ-Carrageenan from CP Kelco U.S., Inc.;
84 Pancreatic α-amylase (A3306) from Sigma-Aldrich (USA). The specifications of the pectin include
85 galacturonic acid (GA) = 65.59±0.57%, degree of methoxylation (DM) = 28.28±0.77%, degree of
86 amidation (DA) = 20.63±0.51%, molecular mass (M_w) = 786±76 kDa. The glucose oxidation kit
87 (GOPOD) was purchased from Megazyme (Ireland). Other chemical reagents were of analytical
88 reagent.

89 2.2 Processing of extruded rice starch (MERS) with pectin (PE) or κ-carrageenan
90 (CG)

91 MERS/PE or MERS/CG was prepared following our previous work (He et al., 2020) but with
92 addition of PE or CG (2.5%, 5%, 7.5%, 10%, wt/wt, based on rice starch) instead. The moisture
93 content of the samples was adjusted to 40% moisture content. Extrusion processing was undertaken
94 using a Haake MiniLab II 40-mm co-rotating twin-screw micro-extruder (Thermo Fisher, USA), as

shown in **Fig. 1**. The reliability of this equipment in wide polymer processing research has been well demonstrated (Wang et al., 2014). The screw speed was set to be 150 rpm and the barrel temperature (only one temperature zone) was 85 °C. 5 g of the sample was fed into the extruder at the feeding port and the extrudate was collected at the exit opening without reflux. The residence time was about 5 min. (**Fig. 1**). The two types of composite prepared were named MERS/PE-X and MERS/CG-X respectively, where the letter "X" stands for the addition of PE or CG (wt%). The MERS sample without PE or CG (control) was named MERS/0, which was characterized previously (He et al., 2020). The term "MERS" is used to describe extruded rice starch (without or with an NSP) in general.

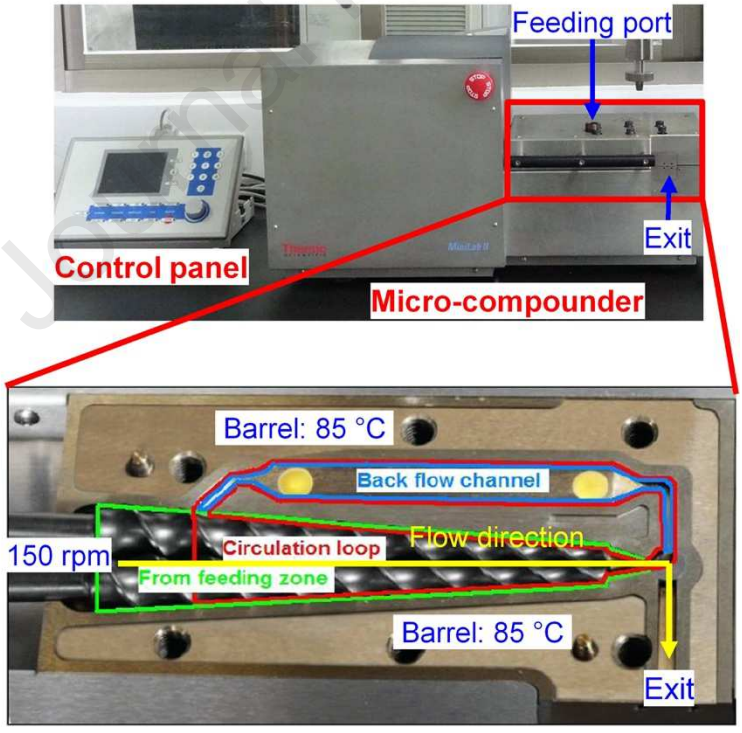

Fig. 1. Pictures of HAAKE MiniLab Micro-extruder and the inside configuration. The yellow line indicates the flow direction used in this work (the backflow channel was not used).

2.3 Characterization and statistical analysis

The samples were characterized and statistically analyzed using the same methods as reported before (He et al., 2020).

3 Results and discussion

3.1 Effects of PE/CG on the digestibility and predicted glycemic index (pGI) of MERS

Table 1 shows that inclusion of PE or CG remarkably inhibited the digestion of MERS. Specifically, with a higher content of PE or CG in the formulation, the RDC content became lower, the resistant component (RC) content higher, and pGI was also reduced. CG seems to be more effective than PE to reduce the digestibility of MERS. Only 2.5% addition of CG could significantly reduce the rapidly-digestible component (RDC) and slowly-digestible component (SDC) contents and predicted glycemic index (pGI) and increase the RC content of MERS. Compared with MERS/PE, MERS/CG exhibited a higher RC content and lower pGI at the same addition amounts (2.5–10%). However, MERS/PE-10% presented even a lower RDC content than MERS/CG-10%. Moreover, the addition of PE increased the SDC content whereas CG had a reverse effect. Due to the difference in chemical structure, the ways of interaction between PE or CG and starch molecules could be quite different, which, in turn, may largely affect the composite structure (Mahmood et al., 2017). A branched biopolymer generally presents a lower viscosity than a linearly structured biopolymer (Xie et al., 2009). The lower viscosity of PE could reduce the overall viscosity of the starch/PE blend, so that starch could suffer from less shear-induced degradation (Hasjim, Xie, Halley, & Gilbert, 2014). Moreover, PE with lower viscosity could be easier to encapsulate starch domains in the blends, restricting starch gelatinization (Tester et al., 2003) and, thus, reduce starch

digestibility. In contrast, CG with a linear chain structure had a higher viscosity so it is less likely to encapsulate starch domains (Marcotte, Hoshahili, & Ramaswamy, 2001). However, the linear structure could make CG chains to interact with starch chains more effectively, leading to a higher RC content of the complex. The above results showed that PE or CG effectively regulated the anti-digestion performance and pGI of rice starch during extrusion, but the regulation effect was not the same. More specifically, the different structural features between PE and CG could lead to different ways of molecular interaction with rice starch.

Table 1. RDC, SDC, RC and pGI of MERS/0, MERS/PE and MERS/CG samples[*]

Samples	RDC (%)	SDC (%)	RC (%)	pGI
MERS/0[#]	75.2±0.6^{g}	10.3±0.6^{d}	14.5±0.5^{a}	82.3±1.5^{g}
MERS/PE-2.5%	73.2±1.0^{g}	11.8±1.2^{e}	15.0±1.2^{b}	79.1±1.3^{f}
MERS/PE-5%	55.9±0.9^{f}	14.2±0.4^{f}	27.9±1.6^{c}	75.7±0.7^{e}
MERS/PE-7.5%	37.0±0.7^{e}	16.2±0.4^{g}	46.8±1.2^{d}	71.8±2.1^{d}
MERS/PE-10%	29.9±0.8^{a}	17.5±0.9^{h}	52.6±1.1^{e}	70.3±1.5^{d}
MERS/CG-2.5%	40.2±0.6^{d}	4.2±0.2^{c}	55.6±1.1^{e}	67.9±2.1^{c}
MERS/CG-5%	37.9±1.0^{c}	2.7±0.9^{a}	59.4±0.7^{e}	65.6±0.9^{b}
MERS/CG-7.5%	36.5±0.7^{b}	2.3±1.1^{a}	61.2±1.4^{f}	65.0±0.8^{b}
MERS/CG-10%	30.2±1.3^{a}	2.1±0.6^{a}	67.7±1.3^{f}	63.1±1.6^{a}

[*] All data were repeated in triplicates and expressed as mean with standard deviation (SD). Different values of different letters in the same column indicate statistical significance ($p < 0.05$).

[#] Data from reference (He et al., 2020).

3.2 First-order kinetics analysis

The digestion behaviors of MERS/PE and MERS/CG samples could be well fitted to

first-order and log of slope (LoS) plots (R^2 > 0.9, **Fig. 2**). The associated parameters such as the first order rate of digestion (*k*) and the digested components ratio at the end of the reaction (C_∞) are shown in **Table 2.** The MERS/PE composites digested more rapidly within the first 1 h. However, a shorter time was used to reach a plateau for the MERS/CG samples (about 40 min) than for the MERS/PE samples (about 60 min). Moreover, the digestion of MERS/PE and MERS/CG samples was a single-phase process (Butterworth, Frederick, Terri, Hamung, & Peter, 2012; He et al., 2020). Compared with those for MERS/PE and MERS/CG samples, *k* for MERS/0 was significantly higher. However, the MERS/CG samples had lower *k* than the MERS/PE samples. *k* is controlled by the catalytic properties of the amylase itself, namely the catalytic rate constant (Roder et al., 2009; Slaughter, Ellis, & Butterworth, 2001). It has been suggested that as digestion proceeds, a low *k* value reflects slow diffusion of amylase into the starch granules (Dhital, Shrestha, & Gidley, 2010). Moreover, the difference in the digestion rate of starch mainly depends on C_∞, namely, the total amount of available/digestible starch. The addition of PE or CG led to notably decreased C_∞. The MERS/CG samples had much lower C_∞ than the MERS/PE samples. This result corresponds to the digestion properties (**Table 1**).

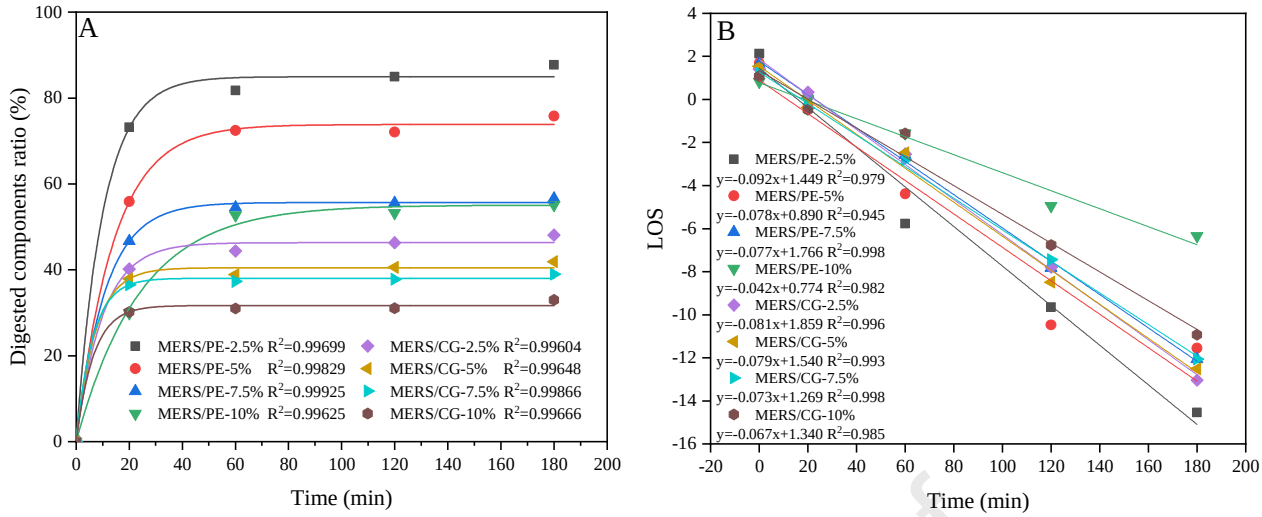

Fig. 2. First-order plots (A) and LOS plots (B) for MERS/PE and MERS/CG samples.

Table 2. k (min^{-1}) and C_∞ (%) for MERS/0, MERS/PE and MERS/CG samples*

Samples	k (min^{-1})	C_∞ (%)
MERS/0#	0.122±0.003^{e}	83.169±1.104^{g}
MERS/PE-2.5%	0.092±0.008^{d}	82.961±1.379^{g}
MERS/PE-5%	0.078±0.011^{c}	73.826±0.926^{f}
MERS/PE-7.5%	0.077±0.002^{c}	57.155±1.308^{e}
MERS/PE-10%	0.042±0.003^{a}	53.729±0.586^{e}
MERS/CG-2.5%	0.081±0.004^{d}	46.331±0.863^{d}
MERS/CG-5%	0.079±0.002^{c}	40.476±0.709^{c}
MERS/CG-7.5%	0.073±0.005^{c}	38.035±0.412^{b}
MERS/CG-10%	0.067±0.002^{b}	31.686±0.542^{a}

* All data were repeated in triplicates and expressed as mean with standard deviation (SD). Different values of different letters in the same column indicate statistical significance ($p < 0.05$).

Data from reference (He et al., 2020).

3.3 Effect of PE/CG on the morphology of MERS

The morphology was investigated by scanning electron microscopy (SEM) as shown in **Fig. 3**. Our previous study (He et al., 2020) showed that the granules of MERS presented irregular shapes and a rough surface, with pores on the surface. This indicates that thermomechanical treatment significantly destroyed the granule structure of native rice starch (NRS). The size of MERS became more homogeneous, ranging from 80 μm to 150 μm. In contrast, MERS/PE or MERS/CG particles had a smooth surface without obvious pores, suggesting that the interactions between PE/CG and rice starch during extrusion led to a homogenous phase on the macroscale and a more compact structure. This structure may inhibit the diffusion of amylase into the interior of the particles. The MERS/PE and MERS/CG samples exhibited similar morphologies.

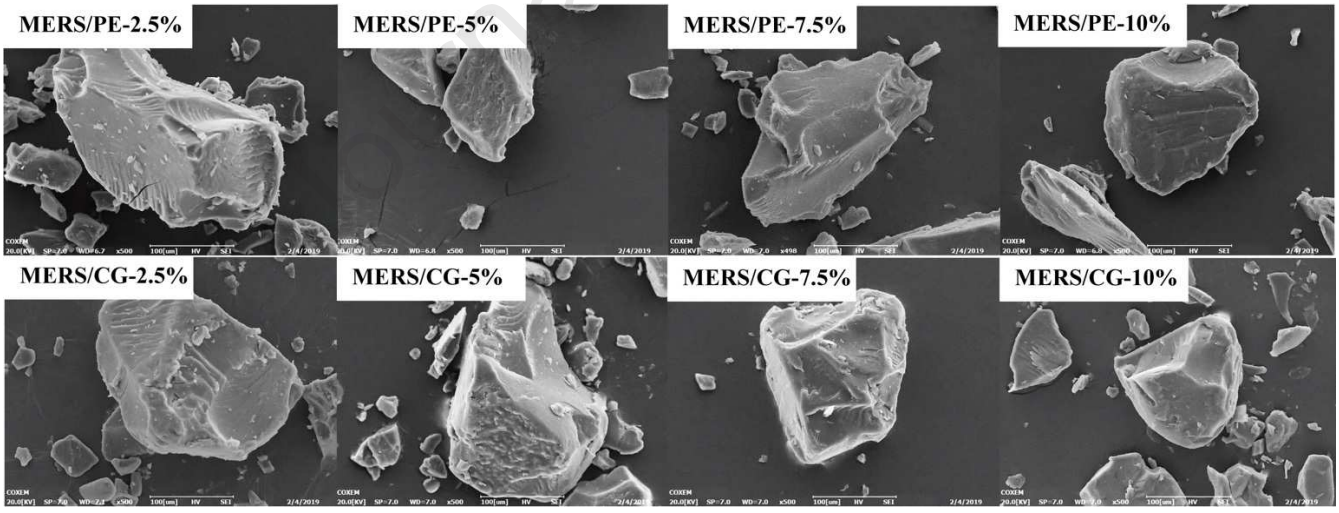

Fig. 3. SEM images of MERS/PE and MERS/CG samples (Scale bar: 100 μm; magnification: 500×).

3.4 Effect of PE/CG on the aggregated structure of MERS

The double-logarithmic small-angle X-ray scattering (SAXS) patterns for the different samples are shown in **Fig. 4**. NRS displayed a typical scattering peak at q of 0.614 nm^{-1}, which disappeared for all the MERS samples including MERS/0 (He et al., 2020). The MERS/PE and MERS/CG samples exhibited a higher scattering intensity than MERS/0 in a small q range, indicating the formation of complexes through interaction between rice starch and PE/CG during extrusion. The fractal dimensions for the MERS/PE and MERS/CG samples with different PE or CG contents were calculated from the slopes of the linear curves in the power-law relationship $I(q) \sim q^{\alpha}$ (Fan et al., 2014; Li, Senesi, & Lee, 2016). The |α| value of the two types of composite (**Table 3**) was in the range from 1 to 3, suggesting the formation of a mass fractal dimension characteristics of fractal structure (D_m) (Suzuki, Chiba, & Yarno, 1997; Zhang et al., 2014). A higher content of PE or CG caused a significant increase in α for MERS, meaning that a denser aggregated structure resulted from the molecular interaction between PE/CG and rice starch. At the same content of addition, the MERS/CG composites exhibited a higher α value than the MERS/PE samples. Given this, linear CG chains were more likely to interact with starch chains leading to a higher degree of molecular aggregation.

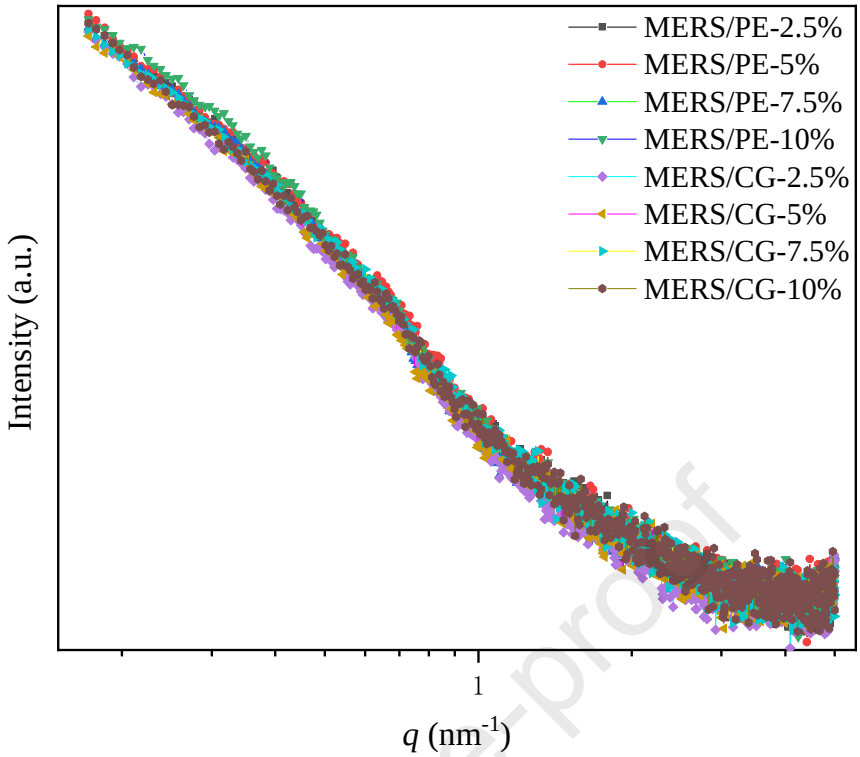

Fig. 4. Double-logarithmic SAXS patterns for MERS/PE and MERS/CG samples.

Table 3. α, total crystallinity (X_{Total}), A+B-type crystallinity (X_{A+B}), V-type crystallinity (X_V), and $R_{1045/1022}$ of MERS/0, MERS/PE and MERS/CG samples. *

Samples	α	X_{Total} (%)	X_{A+B} (%)	X_V (%)
NRS#	1.66±0.02^a	32.4±0.5^f	32.0±0.4^f	0.4±0.1^a
MERS/0	2.10±0.03^b	16.6±0.13^a	15.4±0.14^b	1.2±0.12^b
MERS/PE-2.5%	2.12±0.01^c	18.2±0.17^b	16.8±0.18^c	1.4±0.12^c
MERS/PE-5%	2.15±0.02^d	18.8±0.13^c	17.2±0.14^d	1.6±0.12^d
MERS/PE-7.5%	2.22±0.03^e	20.1±0.19^d	18.3±0.16^e	1.8±0.17^e
MERS/PE-10%	2.23±0.02^f	20.9±0.18^d	18.7±0.16^e	2.1±0.16^f
MERS/CG-2.5%	2.21±0.03^e	20.0±0.17^d	15.0±0.18^b	5.0±0.12^g
MERS/CG-5%	2.24±0.01^g	20.8±0.13^d	14.5±0.14^a	6.3±0.12^h
MERS/CG-7.5%	2.25±0.03^g	21.2±0.19^e	14.6±0.16^a	6.8±0.17^i
MERS/CG-10%	2.26±0.02^h	21.9±0.18^e	14.2±0.16^a	7.7±0.16^j

206 * All data were repeated in triplicates and expressed as mean with standard deviation (SD). Different values of
207 different letters in the same column indicate statistical significance ($p < 0.05$).
208 # Data from reference (He et al., 2020).
209

210 3.5 Effect of PE/CG on the crystalline structure of MERS

211 **Fig. 5** shows the X-ray diffraction (XRD) patterns for the different samples. The peaks of
212 MERS/0 appeared at 15°, 17°, 20°, and 23.3° (2θ), suggesting an A+B+V hybrid crystalline structure
213 (He et al., 2020). Compared with NRS (He et al., 2020), all the MERS samples had low crystallinity.
214 Given this, the extrusion process had largely destroyed the original crystalline structure and caused
215 some extents of gelatinization of these samples. Compared with MERS/0, the samples added with PE
216 or CG presented the same type of crystalline structure but slightly higher crystallinity (**Table 3**).
217 These results indicate that addition of PE or CG probably inhibited gelatinization during the
218 thermomechanical processing. CG dramatically increased the V-type crystallinity (X_V) of MERS
219 whereas there were no apparent changes to the A+B-type crystallinity (X_{A+B}). In comparison, PE
220 gradually increased the X_{A+B} of MERS with increasing content but only significantly moved X_V to a
221 much higher value when the addition amount was 10%. As a result, the MERS/CG samples only
222 showed slightly higher X_{Total} than the MERS/PE samples. During extrusion, starch undergoes a phase
223 transition at a low moisture content, which disrupts its crystalline structure and semi-crystalline
224 lamellae (Chen, Zhu, & Liu, 2017; Liu et al., 2017; Zhang et al., 2014). Previous studies reported
225 that extrusion led to the destruction of the original A-type crystal structure of sweet potato and its
226 reorganization to form a weak B-type crystal structure (Van Soest, Hulleman, De Wit, & Vliegenthart,
227 1996; Waramboi, Gidley, & Sopade, 2014). NSPs such as PE and β-glucan may adhere to the surface

of starch particles through van der Waals forces or hydrogen bonding under extrusion treatment, which inhibits the ordered structure destruction, resulting in higher X_{A+B} (Brennan, Derbyshire, Tiwari, & Brennan, 2013; Robin, Schuchmann, & Palzer, 2012; Wang, Jin, & Yuan, 2007). CG might not have such a strong effect due to its linear chain structure and higher viscosity, although it could promote the growth of V-type crystallites.

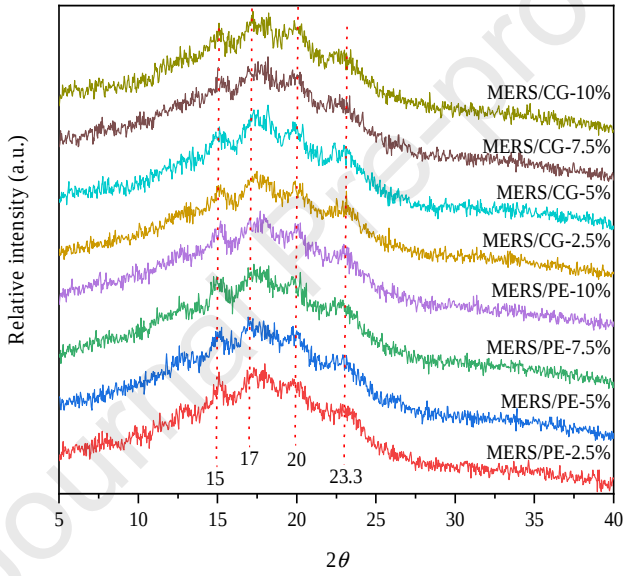

Fig. 5. X-ray diffractograms for MERS/PE and MERS/CG samples.

3.6 Effect of PE/CG on the helical structure of MERS

The solid-state ^{13}C cross-polarized magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectra for NRS and the MERS/PE and MERS/CG samples are shown in **Fig. 6**. The peak at 99–102 ppm in the C_1 region indicates V-type single helices, while the peak at 103.2 ppm is related to amylopectin double helices and amorphous structure (Gidley & Bociek, 2002; Morrison, Tester,

242 Gidley, & Karkalas, 1993). Accordingly, the contents of double helices, single helices and
243 amorphous structure can be calculated (Gidley et al., 2002; Morrison et al., 1993), which are listed in
244 **Table 4**. Compared with NRS, the MERS samples exhibited higher contents of single helices and
245 amorphous starch but a lower content of double helices. Compared with MERS/0 (He et al., 2020),
246 both MERS/PE and MERS/CG samples showed a remarkably reduced content of amorphous starch.
247 Nonetheless, the MERS/PE samples presented both significantly higher contents of single helices
248 and double helices whereas CG notably increased the single helices content but reduced the double
249 helices content in MERS. Likely, the branched chains of PE can interact with rice starch to form new
250 double helices through hydrogen bonding (Naqash et al., 2017). In contrast, CG with a linear
251 molecular structure tends to interact with starch by hydrogen bonding between their main chains. As
252 both CG and amylose tend to form a helical structure, one may form single helices incorporating the
253 other (Lascombes et al., 2017). Moreover, the thermomechanical treatment broke α-1,4 and α-1,6
254 glycosidic bonds, resulted in more amylose content and the production of more single helical
255 structure (Liu et al., 2017; Van Soest et al., 1996). The susceptibility of polymer chains to shear
256 degradation is affected by the branch structure; a shorter branch length and higher branch density are
257 related to higher sensitivity to shear degradation (Liu, Halley, & Gilbert, 2010). PE with a branch
258 structure tends to undergo chain scission under shear treatment. This could reduce the shear effect on
259 the starch, leading to a higher double-helix content. However, this protection effect was much less
260 apparent when linearly structured CG was used.
261

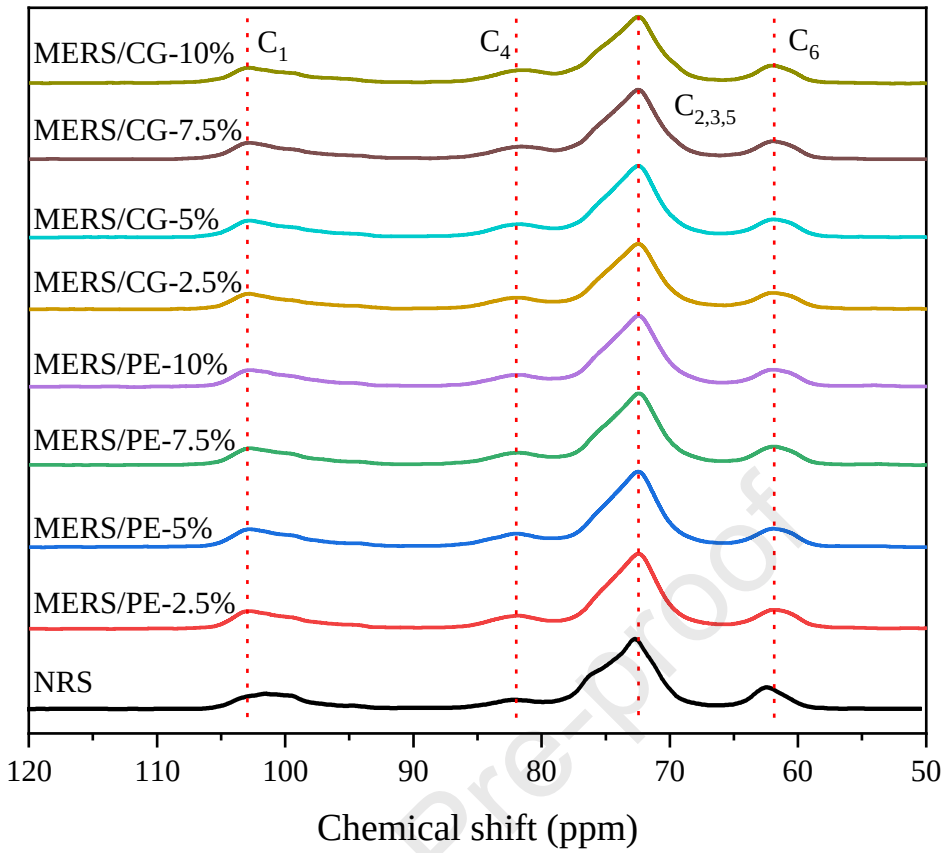

Fig. 6. ^{13}C CP/MAS NMR spectra for MERS/PE samples, MERS/CG samples, and NRS.

Table 4. Percentages of single helices, double helices and amorphous starch in MERS samples and NRS measured by ^{13}C CP/MAS NMR.*

Samples	Amorphous	Single helix	Double helix	$R_{1045/1022}$
NRS	27.7±1.3^{a}	1.8±0.9^{a}	70.5±0.4^{g}	0.773±0.004^{a}
MERS/0$^{\#}$	79.2±0.5^{e}	2.5±0.3^{b}	18.3±0.8^{d}	0.815±0.002^{b}
MERS/PE-2.5%	78.7±0.4^{e}	2.9±0.1^{b}	18.4±0.8^{c}	0.878±0.004^{f}
MERS/PE-5%	77.7±0.1^{c}	3.4±0.0^{c}	18.9±0.5^{d}	0.882±0.012^{g}
MERS/PE-7.5%	76.0±0.5^{c}	4.6±0.3^{d}	19.4±0.1^{e}	0.887±0.003^{g}
MERS/PE-10%	74.5±0.0^{b}	4.9±0.0^{d}	20.6±0.0^{f}	0.894±0.016^{h}
MERS/CG-2.5%	76.8±0.4^{c}	5.6±0.1^{e}	17.6±0.5^{b}	0.825±0.014^{c}
MERS/CG-5%	77.0±0.5^{d}	6.4±0.3^{f}	16.6±0.8^{a}	0.832±0.012^{d}

MERS/CG-7.5%	76.8±0.0^{c}	7.8±0.0^{f}	15.4±0.0^{a}	0.859±0.003^{e}
MERS/CG-10%	76.6±0.4^{c}	8.1±0.1^{f}	14.3±0.5^{a}	0.877±0.016^{e}

[*] All data were repeated in triplicates and expressed as mean with standard deviation (SD). Different values of different letters in the same column indicate statistical significance ($p < 0.05$).

[#] Data from reference (He et al., 2020).

3.7 Effect of PE/CG on short-range order of MERS

The changes of short-range order in starch was investigated by Fourier-transform infrared (FTIR) spectroscopy (Van Soest, Tournois, De Wit, & Vliegenthart, 1995; Warren, Gidley, & Flanagan, 2016). The ATR-FTIR spectra for NRS and the MERS/PE and MERS/CG samples are shown in **Fig. 7**. The calculated $R_{1045/1022}$ values are listed in **Table 4**. A higher $R_{1045/1022}$ value indicates higher amounts of short-range ordered structure (Soest, Tournois, Wit, & Vliegenthart, 1995; Warren et al., 2016). Compared with NRS, the MERS samples resulted in significant higher $R_{1045/1022}$. The data shows that the inclusion of PE or CG enhanced short-range order in both types of composite, with a higher amount of addition providing a better effect as compare to MERS/0 (He et al., 2020). The effect of PE was shown to be more pronounced, which may be due to its lower viscosity (Marcotte et al., 2001) and, thus, greater effect to assist short-range structural ordering in starch. This result corresponds to the NMR data (**Table 4**).

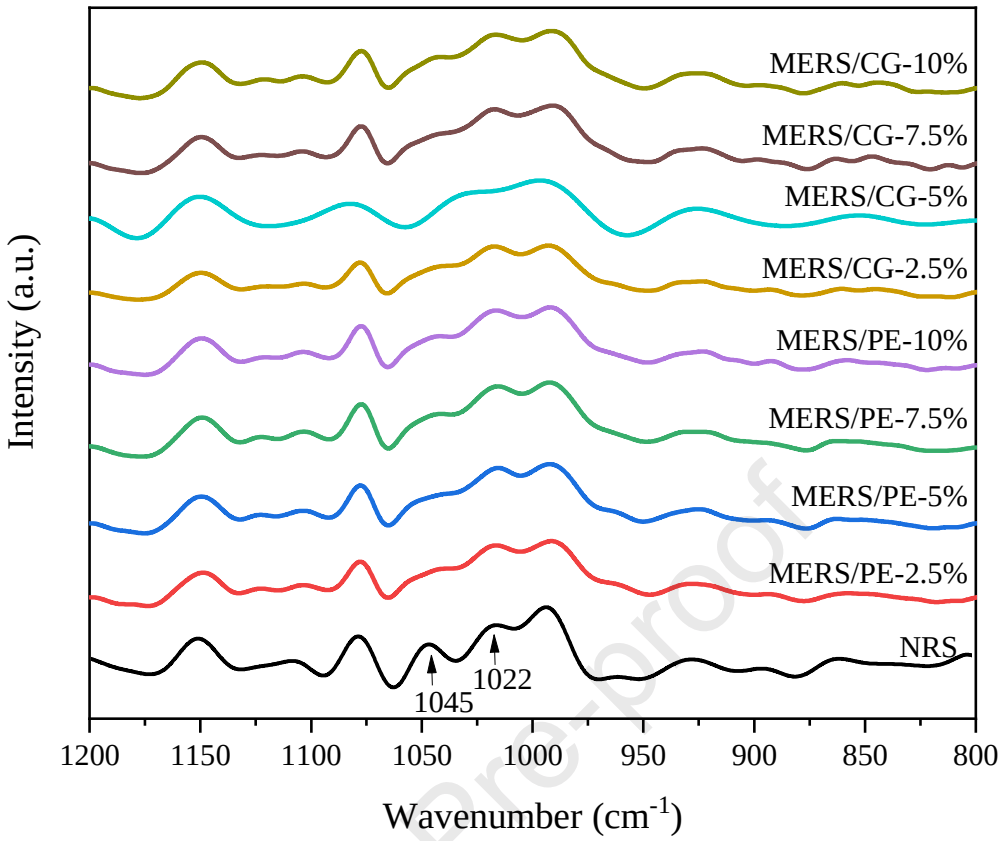

Fig. 7. FTIR spectra for MERS/PE, MERS/CG samples and NRS.

3.8 Correlation between PE/CG addition and the structure and digestibility of MERS

Table 5 shows the Pearson correlation coefficients calculated for the relationship among the PE/CG content, MERS structure and digestibility.

For the MERS/PE samples, the RDC content significantly negatively correlates with the RC content and SDC content; the SDC and RC contents significantly negatively correlate with amorphous starch content and pGI, and significantly positively correlate with α, X_{Total}, X_{A+B}, X_V, single-helix content, double-helix content, and $R_{1045/1022}$. This indicates that the enhancement of these structural features increases the SDC and RC contents, following the sequence of X_{Total} > X_{A+B} > X_V > single-helix content > α > $R_{1045/1022}$ > double-helix content. The PE addition amount shows a

296 significant negative correlation with RDC content, amorphous starch content, and pGI; and a
297 significant positive correlation with RC, α, X_{Total}, X_{A+B}, X_V, $R_{1045/1022}$, single-helix content, and
298 double-helix content. This indicates an increasing amount of PE addition would promote the
299 structural ordering and, thus, digestion resistance. The PE addition influences these structural
300 features in the sequence of X_{Total} > $R_{1045/1022}$ > X_{A+B} > single-helix content > X_V > double-helix
301 content.

302

303 **Table 5.** Pearson correlation coefficients for the relationship among PE/CG content, MERS structure
304 and digestibility. *

	MERS/PE				MERS/CG			
	content	RDC	SDC	RC	content	RDC	SDC	RC
PE or CG content	1	–	–	–		–	–	–
RDC	−0.994**	1	–	–	−0.947	1	–	–
SDC	0.992**	−0.985*	1	-	−0.910	0.777	1	–
RC	0.983*	−0.961*	0.992**	1	0.973*	−0.993*	−0.846	1
α	0.956*	−0.966*	0.981*	0.954*	0.964*	−0.854	−0.991*	0.910
X_{Total}	0.996**	−0.982*	0.995**	0.995**	0.993**	−0.950*	−0.932	0.980*
X_{A+B}	0.979*	−0.951*	0.974*	0.991**	−0.899	0.903	0.905	−0.935
X_V	0.973*	−0.984*	0.986*	0.958*	0.985*	−0.929	−0.954*	0.966*
$R_{1045/1022}$	0.992**	−0.987*	0.967*	0.957*	0.979*	−0.935	−0.820	0.946
Amorphous starch	−0.995**	0.981*	−0.978*	−0.977*	−0.632	0.734	0.258	−0.670
Single helices	0.974*	−0.944*	0.977*	0.996**	0.980*	−0.858	−0.913	0.968*
Double helices	0.972*	−0.975*	0.935*	0.916*	−0.999**	0.947	0.899	−0.971*
pGI	−0.986*	0.970*	−0.977*	−0.999**	−0.979*	0.945	0.938	−0.977*

305 *p < 0.05 and **p < 0.01 indicate statistical significance.

306

For the MERS/CG samples, the content of RDC significantly negatively correlates with RC content. The RC content shows a significant positive correlation with X_{Total}, X_V, and single-helix content and a significantly negative correlation with the content of double helix and pGI. The influence of these structural features on RC content follows the sequence X_{Total} > single-helix content > X_V. The content of CG addition demonstrates a significant positive correlation with RC content, α, X_{Total}, X_V, $R_{1045/1022}$ and single-helix content, and an obvious negative correlation with the content of double helix and pGI. This indicates that a higher content of CG added increases the digestion resistance by promoting the formation of the crystalline and short-range ordered structures. The CG addition positively influences the different structural features in the sequence of X_{Total} > X_V > single-helix content > $R_{1045/1022}$ > α and reduces the double-helix content.

3.9 Discussion on mechanism regarding the digestion resistance of MERS/PE and MERS/CG

Fig. 8 is a schematic representing the evolution of the multiscale structural of thermomechanically-processed rice starch with PE or CG.

The different molecular structures of PE and CG could make their interactions with starch different. The PE has a branched-chain structure and may inhibit the proximity between the main chains of PE and starch (Luo, Chen, Li, Liang, & Chen, 2017). The interaction between starch and PE mainly occurs via their side chains by hydrogen bonding and entanglement. As a result, new double helices and A+B-type crystallites (higher X_{A+B}) are formed. Owing to the steric hindrance effect, it is difficult for PE chains to be wrapped by amylose to form single-helices or V-type crystallites (Sajjan & Rao, 1987; Sudhakar, Singhal, & Kulkarni, 1996). In addition, owing to its

lower viscosity (Marcotte et al., 2001; Sudhakar et al., 1996), PE tends to increase the mobility of starch chains (especially side chains of amylopectin), resulting in a higher degree of molecular order ($R_{1045/1022}$) and enhanced aggregated structure (α) of MERS/PE composites. Moreover, the lower viscosity of PE may also allow it to encapsulate starch domains in the blends. All these structural changes could provide a shielding effect on the binding site of starch chains against amylase. In this way, the hydrolysis rate and degree of hydrolysis of rice starch could be reduced with higher contents of SDC and RC.

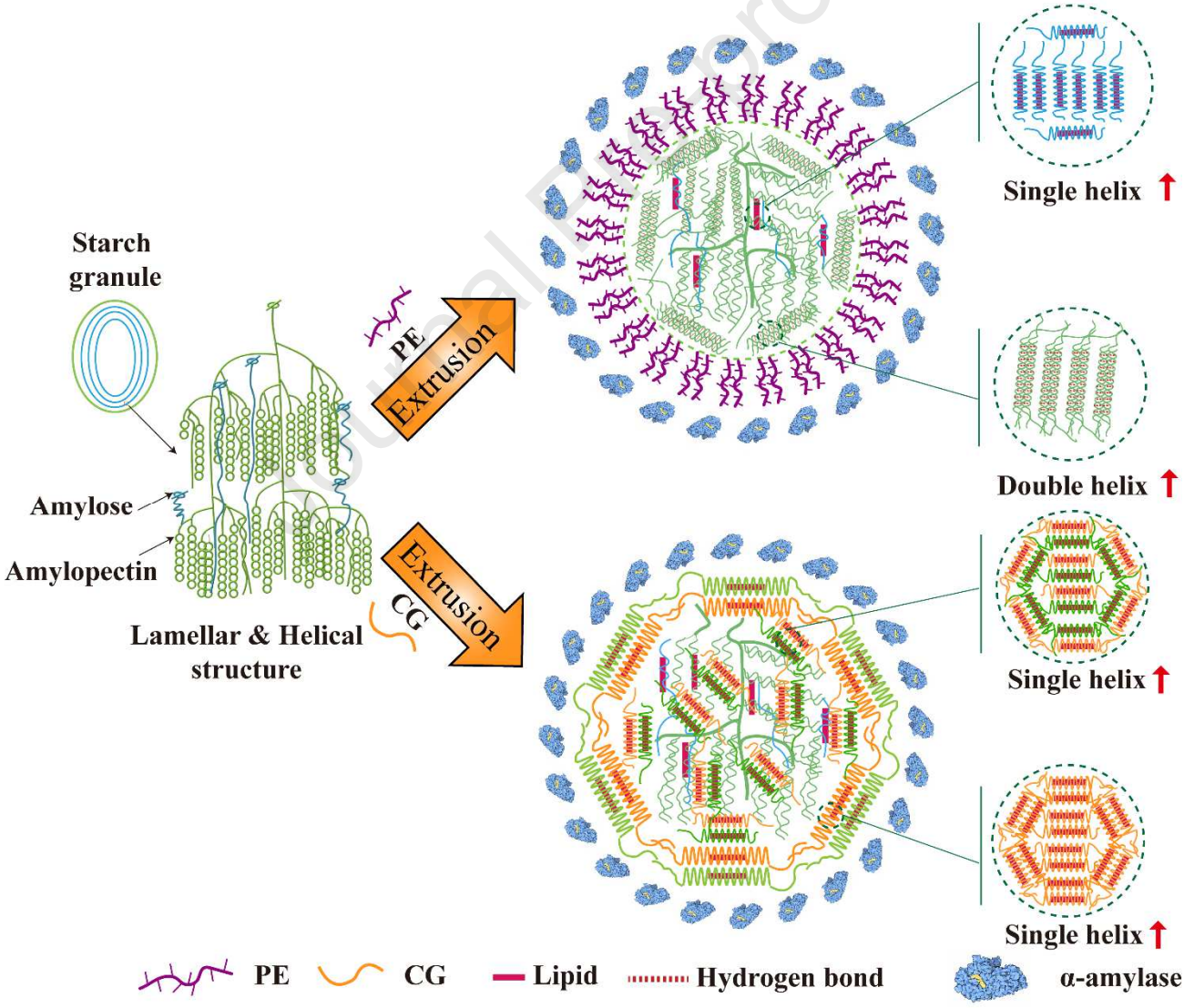

Fig. 8. Schematic representation of the alterations in the multiscale structure and the digestibility of

MERS/PE and MERS/CG.

CG has a linear molecular structure so it can interact with starch through hydrogen bonding more effectively via its main chain. This interaction during extrusion may also disrupt the original double-helical structure and destroy the original A+B-type crystallites (lower X_{A+B}). The interaction also contributes to a greater structural ordering including the formation of new molecular aggregates, single helices, and V-type crystallites (with amylose), which can effectively shield the binding sites of starch chains for amylase and restrict starch hydrolysis. Even CG with low addition could effectively interact with starch to increase the RC content of MERS significantly, with a reduction in SDC content.

4 Conclusion

This study has demonstrated the improved digestion resistance (higher RC and lower pGI) of MERS/PE and MERS/CG composites prepared by thermomechanical treatment. A relevant model is established and the mechanism is elucidated. PE has a branched-chain structure, could only form hydrogen bonding with starch via its side chains, leading to higher contents of double helices and A+B-type crystallites (higher X_{A+B}), and higher degrees of molecular aggregation (α), and short-range order ($R_{1045/1022}$). All these structural changes caused increases in SDC and RC contents. On the other hand, CG, which has a linear chain structure, can interact with starch via its main chains by hydrogen bonding. Therefore, CG is more effective at increasing the structural ordering of MERS, which has higher contents of single helices, V-type crystallites (higher X_V), and leading to higher RC content. The multiscale structural changes of starch due to the addition of NSPs such as PE and CG

during extrusion could effectively shield the action sites on starch chains for amylase and restrict the interaction of amylase with starch, leading to a higher RC content and lower pGI.

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Conflicts of interest

There are no conflicts of interest to declare.

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537

Highlights

- ✓ Extruded rice starch (MERS) was prepared with pectin (PE) or κ-carrageenan (CG)
- ✓ Both MERS/PE and CG showed higher resistant components (RC) content
- ✓ Both MERS/PE and CG presented lower predicted glycemic index (pGI)
- ✓ Crystallinity and helical structures contributed to SDC and RC in MERS/PE
- ✓ More single helices and V-type crystallites (X_V) led to higher RC in MERS/CG

– Declaration of Interest –

Different effects of pectin and κ-carrageenan on the multiscale structures and *in vitro* digestibility of extruded rice starch

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The authors declare that there is no conflict of interest regarding the publication of this article.