Supplementary Information

Preparation of a novel expandable konjac fiber under different freezing temperatures and exploration of its digestion regulation functions

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2. Materials and methods

2.1 Chemical composition analysis

The moisture content was measured using an HE53/02 rapid moisture meter (Mettler Toledo, Shanghai, China). The protein content was measured according to standard Kjeldahl methodology, and the nitrogen content was multiplied by 6.25. The lipid and ash content of konjac powder and rice grains were measured according to GB 5009.6 and GB 5009.4, respectively. Starch content was measured according to the instructions of the assay kit. Determination of the purity of konjac powder according to the NY/T 494-2010.

2.2 Swelling and water-holding capacity of konjac fiber

The simulated gastric fluid (SGF) consisted of 0.5 mg/mL KCl, 0.1 mg/mL KH_2PO_4, 2.1 mg/mL $NaHCO_3$, 2.5 mg/mL NaCl, 0.02 mg/mL $MaCl_2(H_2O)_6$, 0.02 mg/mL $CaCl_2(H_2O)_2$ and 4000 U/mL pepsin. The pre-weighed konjac fiber was dispersed into the SGF for 90 seconds at 37 °C. At the indicated point, the konjac fiber was withdrawn and weighed. The swelling rate is expressed as follows:

$$SR(\%) = \frac{m_t - m_0}{m_0} \times 100 \quad \text{(S1)}$$

Where m_t(g) refers to the weight of konjac fiber at the time t and m_0(g) is the dry basis.

The water-holding ability of fiber was estimated according to the method of centrifugation. Put the gastric-digested konjac fiber into a cylindrical plastic tube with a perforated bottom and filter paper covered. The tube was centrifuged at 10000 g for 30 min. The fiber weight was recorded after the solution on the surface of the fiber was

removed. The following formula was used.

$$\text{Water holding capacity} = (m_1 - m_0)/m_0 \quad \text{(S2)}$$

Where m_1 refers to the wet weight of the fiber and m_0 is the dry basis of the konjac fiber.

2.3 Water migration in the konjac fiber

The equipment was first calibrated according to the standards provided by the manufacturer. 0.02 g konjac fiber was dispersed in 200 μL water for 1min. The fiber was withdrawn and put into the NMR testing tube (15 mm diameter), which was inserted into the instrument probe. The testing parameters were set as follows: SW = 200 kHz; RG = 20 dB; NECH = 5000; TE = 0.5 ms; NS = 8, TW = 4000 ms. The Carr-Purcell-Meiboom-Gill (CPMG) sequence was used to record the transverse relaxation time(T_2) and the T_2 was recorded by T_{2b}, T_{21}, $T_{22,}$ and $T_{23.}$ The corresponding proton populations, namely, P_{2b}, P_{21}, $P_{22,}$ and P_{23} were recorded.

2.4 Texture measurement

The freshly prepared konjac fiber (15 mm×15 mm×10 mm) was evaluated by texture profile analysis with a stable microsystem (TA-XT plus) Texture analyzer (Stable Micro Systems Ltd., Surrey, England) with a cylindrical probe P36R. The sample was axially compressed to 45% strain under a double compression test with the procedure as follows: pre-test speed:1.0 mm/s; test speed:5.0 mm/s; post-test speed:5.0 mm/s. The trigger force was set as 0.0045 N. And there were 5 s intervals between the two cycles. The texture parameters of the konjac fiber were obtained as follows: Hardness (H) was the peak force (N) according to the first compression.Cohesiveness

(Ch) was the ratio of the area of the curve under the second compression to that done under the first compression curve. Springiness (Sp)was the distance of the sample recovers after the first compression. Gumminess (Gu) can be determined by hardness and cohesiveness (Gu=H×Ch). Chewiness (Cw) was the mathematical product of hardness, cohesiveness, and springiness (Cw=H×Ch×Sp). Resilience means the ability of the product to recover its original height. All the measurements were carried out in triplicate and the parameters were obtained from the equipped Texture Exponent software (Stable Micro System Ltd., Ver 6.1.16.0, UK).

2.5 Volume of konjac fiber before and after gastric digestion

The fiber (before or after gastric digestion) was put into the graduated cylinder bottom with 1 mm standard beads (Wuhan Servicebio Technology Co., Ltd) to the specified value, and recorded the volume data (V_1). After removing the konjac fiber, the volume of the remaining beads was measured (V_2). The total volume of konjac fiber (V) can be calculated by the following formula:

$$V = V_1 - V_2 \quad \text{(S3)}$$

The macroscopic images of fiber before and after gastric digestion were taken by a digital camera.

2.6 The rheology of digesta emptying from the stomach

Steady shear and dynamic oscillatory tests of the gastric emptying chyme were conducted on a stress-controlled rheometer (DHR-2, TA Instruments, USA) with a 60 mm diameter parallel-plate geometry with a 500 μm gap. The procedure was performed at 37 °C which allowed the sample to be equilibrated for 1 min before the test. The

shear rate from 0.1 to 100 1/s was taken on both test modes.

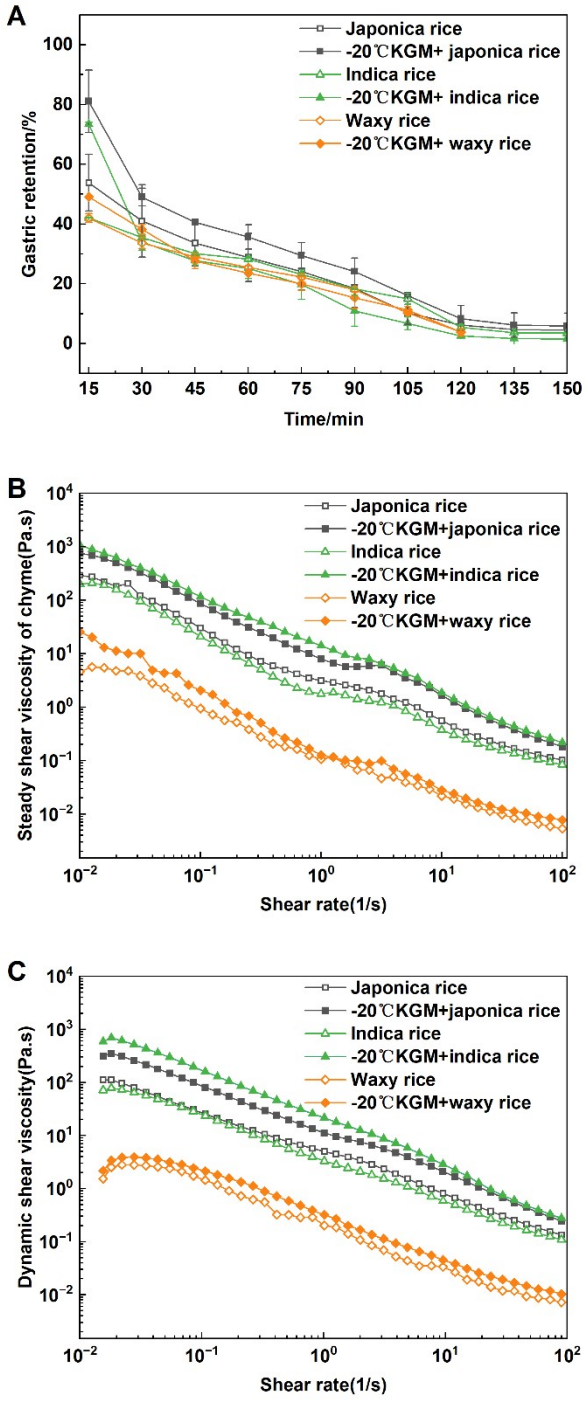


Fig. S1 The effect of -20 °C KGM on gastric emptying and rheology of three different rice varieties.

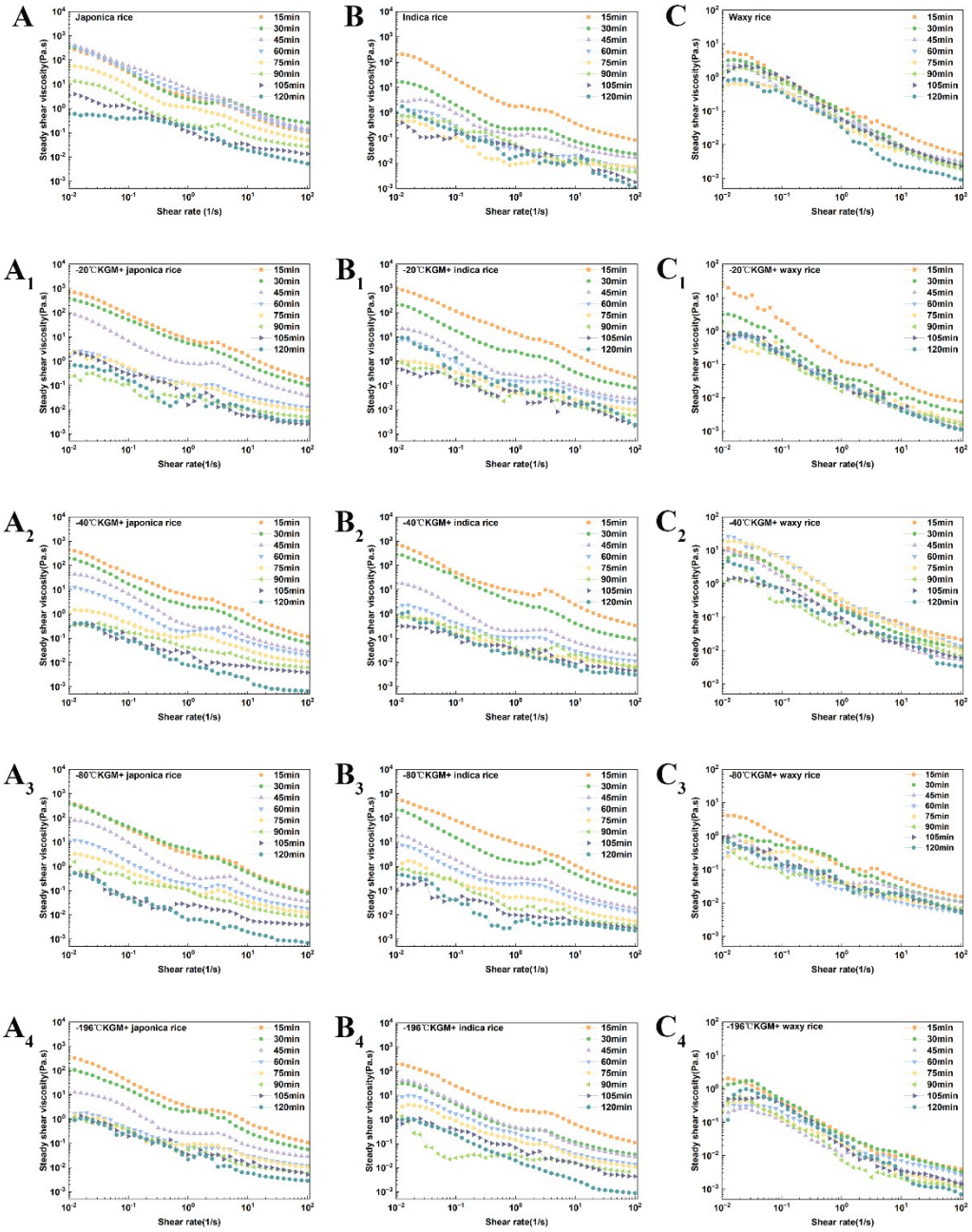


Fig. S2 Steady shear viscosity of gastric emptying chyme with different konjac fiber existence.

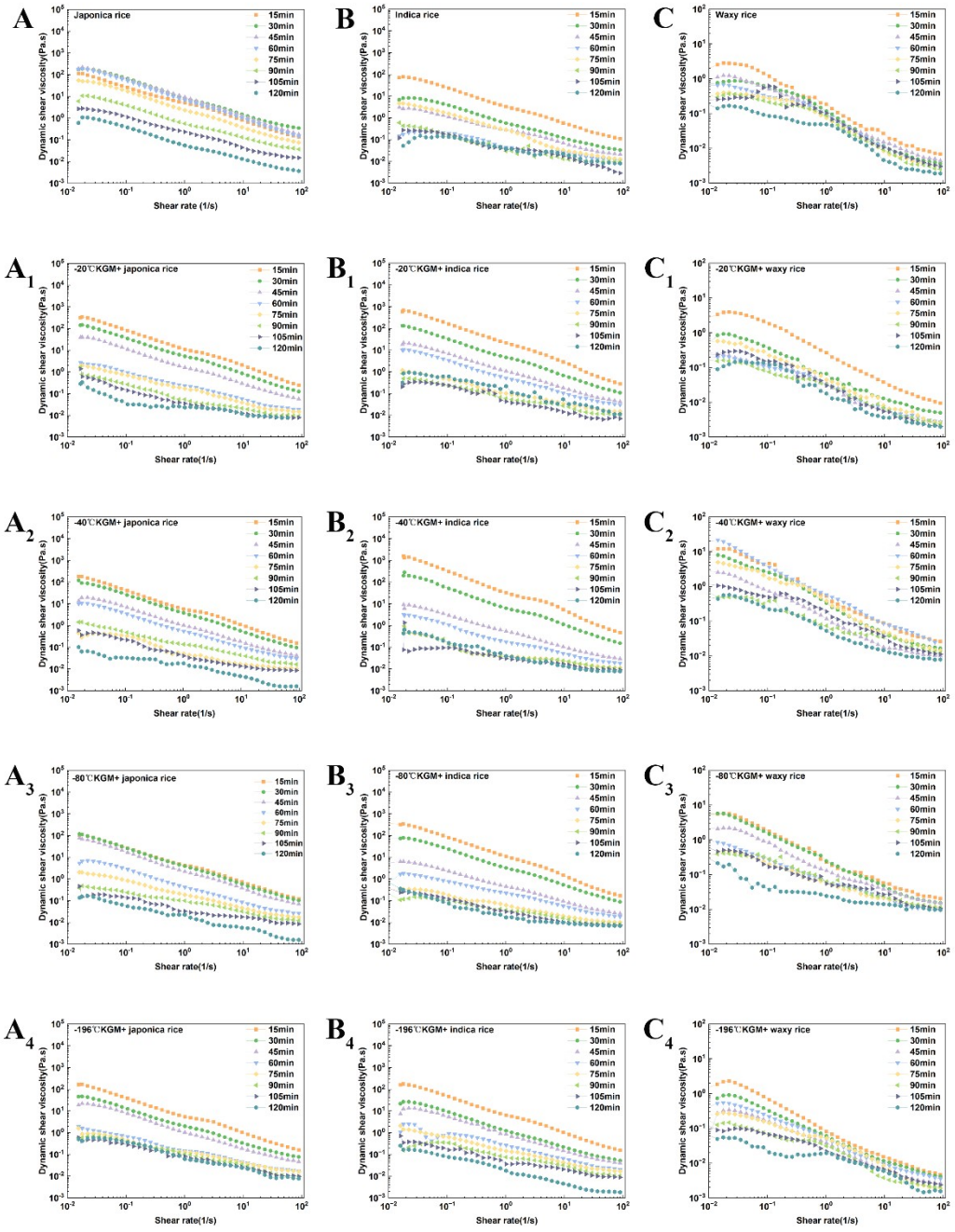


Fig. S3 Dynamic shear viscosity of gastric emptying chyme with different konjac fiber existence.

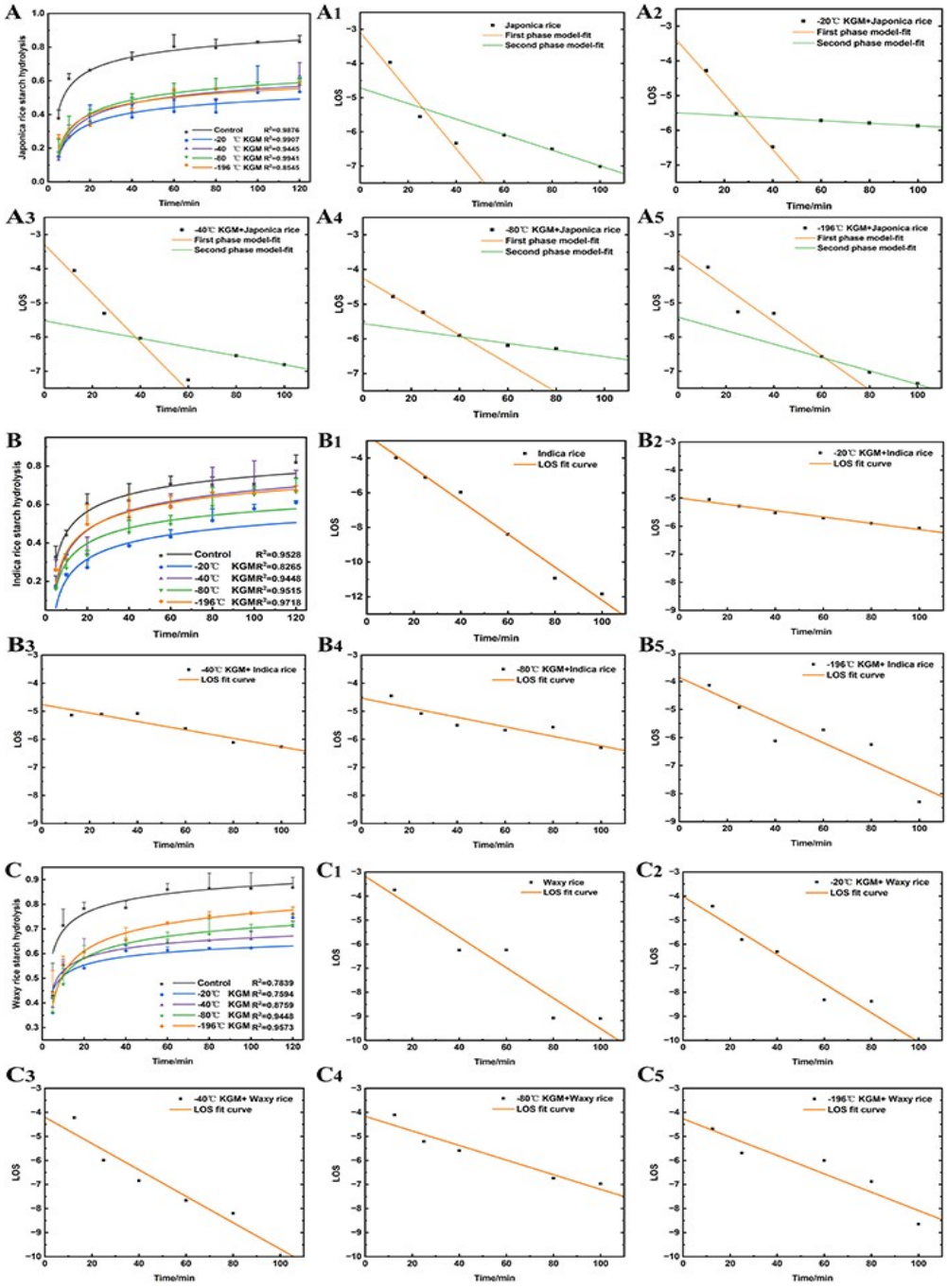


Fig. S4 Digestion profiles of three kinds of rice chyme and LOS plots of rice chyme with different konjac fiber. (A) Japonica starch digestion profiles (A1-A5) LOS plots of japonica rice chyme with (or no) konjac fiber. (A) Japonica starch digestion profiles (A1-A5) LOS plots of japonica rice chyme with (or no) konjac fiber. (B) Indica starch digestion profiles (B1-B5) LOS plots of indica rice chyme with (or no) konjac fiber. (C) Waxy starch digestion profiles (C1-C5) LOS plots of waxy rice chyme with (or no) konjac fiber.

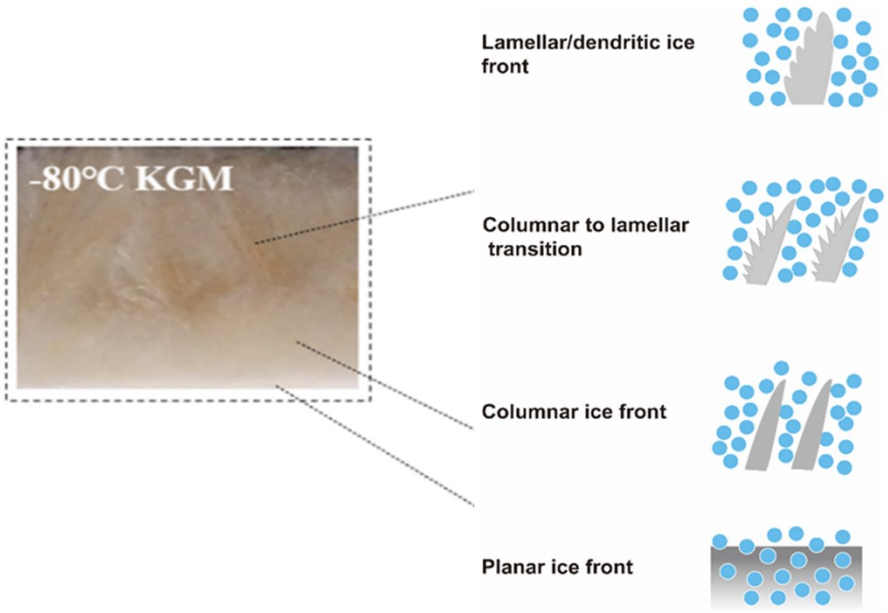


Fig. S5 The cross-section morphology of konjac fiber prepared under -80 °C (left) and the microstructure of the ice front morphology(right). The horizontal cross-sections (parallel to the ice front) reveal the corresponding evolution of the porous structure and hence the interface morphology (depicted on the right). The columnar shapes represent the ice front, and the circle represents a polysaccharide (konjac glucomannan).

Table S1 Chemical composition analysis

Sample	Moisture/ %	Protein/ %	Lipid/%	Ash/%	Starch content/%	Glucomannan content/%
KGM	8.20±0.07	0.76±0.02	0.70±0.06	1.13±0.28	13.13±0.02	76.06±0.05
Japonica rice	9.97±0.05	6.98±0.13	1.01±0.07	0.72±0.06	74.64±0.05	-
Indica rice	10.75±0.08	7.06±0.12	0.95±0.18	0.84±0.01	77.81±0.04	-
Waxy rice	10.88±0.12	6.28±0.17	1.05±0.04	0.80±0.02	70.14±0.04	-

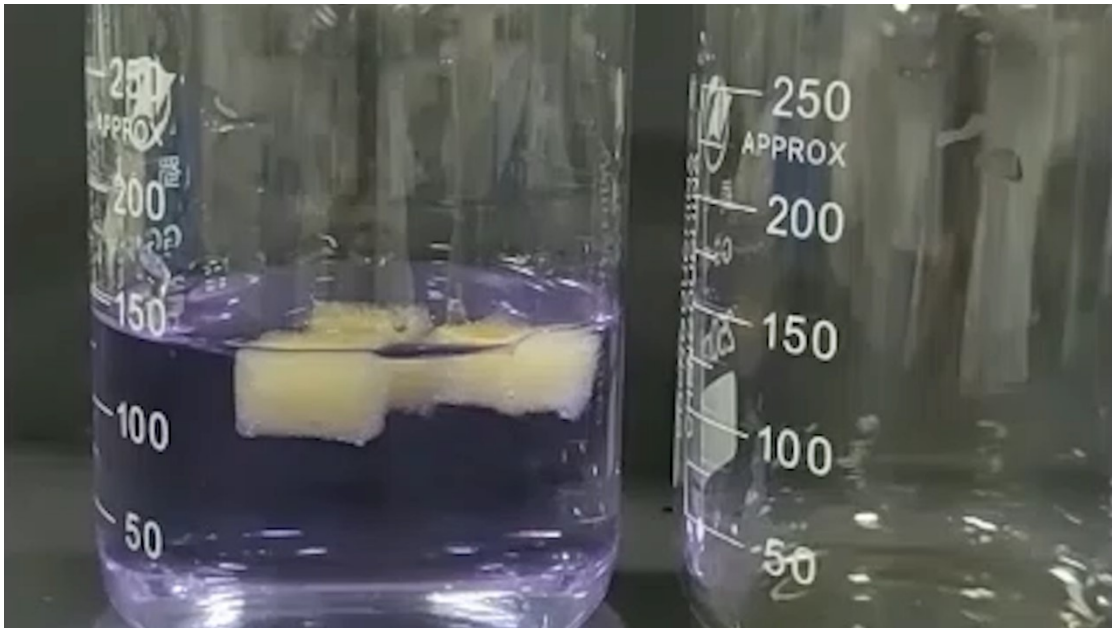
Note: - refers to not determined.

Table S2 Kinetic parameters estimated of japonica rice, indica rice, and waxy rice.

Rice variety	Parameters	Control	-20°C KGM+ rice	-40°C KGM+ rice	-80°C KGM+ rice	-196°C KGM+ rice
Japonica	k_1	-0.08513	-0.07943	-0.07132	-0.01825	-0.04974
rice	k_2	-0.02285	-0.00377	-0.01269	-0.00954	-0.01964
Indica rice	k	-0.09533	-0.01125	-0.01509	-0.01698	-0.03880
Waxy rice	k	-0.06324	-0.06043	-0.05485	-0.03031	-0.03810

Table S3 Kinetic parameters of α-amylase-catalyzed reaction.

Variety		V_{max}(mg/mL/min)	K_m(mg/mL)
	enzyme	0.2024±0.0136	18.4418±2.0787
-20℃	KGM+ enzyme	0.0966±0.0088	15.0145±2.4692
-40℃	KGM+ enzyme	0.1164±0.0044	15.0530±1.0161
-80℃	KGM+ enzyme	0.1176±0.0041	15.1844±0.9384
-196℃	KGM+ enzyme	0.1466±0.0210	15.3838±3.9340



Video S1 The new kind of insoluble konjac fiber can instantaneously expand and exert a space-occupied role to regulate digestion. The cover of the movie was shown below.