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Posted Date: 10 May 2023

doi: 10.20944/preprints202305.0707.v1

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 doi:10.20944/preprints202305.0707.v1

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High Protein Yangyu Jiaotuan: Some Physico-Chemical, Textural, Microstructural, Rheological Properties, and In Vitro Oral-Gastro-Small Intestinal Starch Digestion

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Abstract: Biomimetic foods are expected to have potential health benefits for the management and prevention of chronic diseases, such as diabetes and cardiovascular disease. In the current research, two commercially available and affordable plant proteins (soy protein isolate-SPI and pea protein isolate-PPI) at two levels (5%, 10%) were added to the Yangyu jiaotuan with an objective to develop a product with reduced glycemic properties and high protein content while maintaining its original taste and texture. The results showed that several important textural properties such as hardness and chewiness did not change significantly during the refrigerated storage. The storage modulus G′ increased with refrigerated storage time for different samples, there were significant differences among the five samples (with and without protein addition) with respect to frequency dependence during rheological measurements. The in vitro starch digestion experiments showed that the starch hydrolysis of Yangyu jiaotuan decreased considerably with the increase in PPI content and during refrigerated storage due to starch retrogradation. Protein has protected the microstructure and there was less damage when compared to samples without protein. The bimodal peaks of the particle size distribution curves showed that the newly developed Yangyu jiaotuan contains two different sizes of particles, the smaller particles (~30 μm) corresponded to PPI and starch granules, while the larger particles corresponded to the fragments of gel network of the starch matrix. Based on the above results, Yangyu jiaotuan mixed with pea protein is a convenient potato staple food product, which complies with the biomimetic potato food very well.

Keywords: potato; Yangyu jiaotuan; microstructure; starch digestion in vitro

1. Introduction

One of the current and future strategies for potato processing is "Biomimetic potato foods (BPFs)" which are expected to provide excellent taste, texture, convenience along with superior nutritional attributes [1]. Apart from that, these foods are expected to address all food safety concerns and any issues related to environmental sustainability [2,3]. Biomimetics represents imitation of nature's methods, mechanisms, processes and it provides an inspiration to create processed BPFs with similar structure and functionality like naturally occurring healthy and nutritious foods [2]. It involves a multidisciplinary approach and considers food chemistry, food structure and digestion, food engineering and technologies, and product designing [4,5]. This also helps our newly developed foods to fulfill the future expectations which the consumer is expecting from the food industry regarding food attributes.

For developing biomimetic or "natural-like" foods, it is very important to look at the structure of foods from molecular to the macro level and its relationship with the functional and digestion

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related properties [5,6]. During consumption of foods, these structures are broken down to smaller units in the reverse order during digestion and they ultimately absorbed by our body [7–10]. Apart from this, taste and texture have their importance as these are influenced by the type and level of processing [11]. The understanding and knowledge of fundamental food structure and the changes associated with it during processing, storage helps us to create new dimensions for our foods.

The paste-like mashed potato named Yangyu jiaotuan (洋芋搅团) is very popular in China, especially in the southeast of Gansu Province (Lintao, Wudu, and Huating), this dish use potato as the main ingredient, paste the potatoes with spicy oil, vinegar, and Chinese sauerkraut. Alvarez et al. showed that weak gel behavior for both fresh and frozen/thawed mashed potatoes without and with added SPI together with a significant decrease of system viscoelasticity (G′ and G″) with increasing SPI volume fraction [12]. However, to the best of our knowledge, studies that involved microstructure and digestion properties of mashed potato with SPI are scarce. The aim of the present study was (1) to study some physico-chemical, textural, and rheological properties, and *in vitro* oral-gastro-small intestinal starch digestion of Yangyyu jiaotuan. (2) to study the starch hydrolysis kinetics, microstructural changes, particle size distribution, and thermal properties occurring during simulated gastric and small intestinal digestion. This product has the inspiration from naturally occurring low glycaemic and high protein foods like beans and legumes and we tried to create interaction among starch and proteins to achieve good functionality and nutritional properties. Regarding food application, this newly developed high protein Yangyu jiaotuan can potentially be used as a glycemic control potato product.

2. Materials and Methods

2.1. Materials and sample preparation

The potato was purchased from the Saturday market in Palmerston North of New Zealand, the Nadine variety was used due to its low solid content. After washing and peeling, cut the potato tubers into 3 cm pieces, add water to cover the potato pieces, boiled for 45 min followed by a drain. The cooked potato pieces were mashed with a potato masher, followed by stirring at high speed for 5 mins with a standard kitchen mixer. Take 300 g of mashed potato, add 15.79 g of SPI or PPI powder, mixed with a standard kitchen mixer for an extra 2 mins to prepare a pasta-like mashed potato with 5% SPI or PPI. Besides, 33.33g protein powder was added to 300 g mashed potatoes to make the samples containing 10% SPI or PPI.

2.2. Textural characteristics: texture profile analysis

The textural properties of pure mashed potato (control) and samples mixed with 5% or 10% SPI and PPI were evaluated by carrying out texture profile analysis (TPA) on a Texture Analyzer (TA-XT plus, Stable Micro Systems, Surrey, UK) fitted with a load cell of 5 kg and using a flat probe (61 mm diameter). The test settings were: test speed, 2 mm/s; distance, 10 mm; trigger force, 0.049 N. Samples stored for 1, 3, and 5 days in a 4 ^{o}C refrigerator were cut into 2 cm cubes to obtain the texture parameters of hardness, springiness, cohesiveness, gumminess, chewiness, and resilience, freshly prepared mashed potatoes were too soft to cut into pieces and are not tested.

2.3. Dynamic rheological properties

Dynamic oscillatory tests were conducted in an Anton Par Physica MCR301 controlled stress oscillatory rheometer (Anton Paar GmbH, Germany). The temperature was maintained at (20±0.1) °C, the gap between plates was 1 mm. Frequency sweeps (from 1–100 rad s^{-1}) were performed at settled stress within the linear viscoelastic range. Experimental data were described according to the following equations [13]:

$$G'(\omega)=K'\omega^{n'} \quad (1)$$

$$G''(\omega)=K''\omega n'' \quad (2)$$

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where, G′, a storage modulus (Pa); G′′, loss modulus (Pa); ω, angular frequency (rad s^{-1}); and K′, K′′, n′, n′′, experimental constants.

2.4. Starch digestion in vitro and its kinetics

A three-step digestion procedure was used, simulated salivary fluid (SSF) contained α-amylase, simulated gastric buffer (SGF) contained pepsin, and simulated small intestine buffer (SIF) contained pancreatin, invertase, and amyloglucosidase were prepared according to [14]. SSF, SGF, and SIF were re-warmed to 37 °C before use. For the oral digestion, Yangyu jiaotuan sample contains 6.8 g starch was mixed with SSF at a mass ratio of 1:1 and topped up to 170 g with distilled water. After 2 min of oral digestion, pH was adjusted to 2 using 3 M and 0.5 M HCL, then 25 mL SGF was added to start the gastric digestion. After 30 min, the pH of the digestion system was adjusted to 6.8 using 3 M and 0.5 M NaOH, followed by adding 23 mL SIF to start the small intestinal digestion. Both the volume of HCL and NaOH were recorded for calculating the starch hydrolysis (%).

Starch digestibility was measured by the glucose released after a certain time of simulated digestion. Glucose released was analyzed by GOPOD reagent (Format K-GLUK 07/11, Megazyme International Ireland Ltd, Ireland) and the results were expressed as starch hydrolysis (%) [15]. Samples were taken during the digestion procedure, O_2 refers to 2 min of oral mastication, G0, G15, and G30 were referred to 0, 15, and 30 min of gastric digestion, I0, I5, I10, I15, I30, I90, and I120 corresponded to 0, 5, 10, 15, 30, 90 and 120 min of gastric digestion. Hydrolysis index (HI) and estimated glycaemic index (*e*GI) of the samples were calculated according to the reported method [16], using white bread as a reference.

2.5. Microstructural characteristics

2.5.1. Light microscopy (LM)

Fresh prepared pure mashed potato and samples mixed with 5% or 10% pea proteins were examined with a Leica CME light microscope (Leica Microsystems GmbH, Wetzlar, Germany), the magnifying power was 100×, samples were stained with 1% iodine indicator, the mashed potato microscopy images were processed using the OMAX ToupView software.

2.5.2. Scanning electron microscopy (SEM)

SEM analysis was performed to examine the topographic characteristics of washed and freeze-dried mashed potato particles and coated with gold (SCD 050, Balzers, Liechtenstein), an FEI Quanta 200 FEI Electron Optics scanning electron microscope (Eindhoven, The Netherlands) was used to examine the samples. Approximately 100 g fresh prepared mashed potato was added to a 1 000 mL beaker, add 800 mL of ultrapure water, gently stir and disperse the sample with a glass rod, pour the supernatant when the mashed potato particles were settled (approximately stand for 10 minutes), add 800 mL of water and repeat the cleaning twice. The sediment is collected in a zip plastic bag, immediately immersed in liquid nitrogen quick-freezing, and then freeze-dried to obtain mashed potato particles. Yangyu jiaotuan digesta was also examined using SEM, the same experiment condition was used.

2.5.3. Confocal laser scanning microscopy (CLSM)

The pure mashed potato (control) and samples mixed with 5% or 10% pea proteins were immediately transferred onto Labserv microscope slides (Thermo Fisher Scientific) for CLSM analysis, samples were stained with the fluorescent dye Fast green [0.1% (w/v)] and fluorescein isothiocyanate (FITC) [0.01% (w/v)] for 20 min. Then, the stained sections were observed with a TCS SP5 DM6000B Laser Scanning Confocal Microscope (Leica Microsystems, Wetzlar, Germany). The excitation wavelength of the laser was 561 nm, while the emission wavelength was set to 517 nm for potato starch and 575 nm for pea protein. Samples were loaded on a motorized XY stage, digital image files were recorded at a resolution of 1024×1024 pixels.

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2.6. Particle size distribution

The particle size distribution of Yangyu jiaotuan digesta at different digestion stages was determined according to the reported method [14], using laser diffraction particle size analysis (Mastersizer 2000; Malvern Instruments Ltd., UK). The relative refractive index applied was 1.70.

2.7. Thermal analysis

The thermal properties of the Yangyu jiaotuan digesta were determined using differential scanning calorimetry (DSC) (DSC; TA Q100, TA Instruments, Newcastle, DE). Samples (approximately 5.0 mg, dry basis) were weighed directly in an aluminum pan, and distilled water was added to obtain a starch-water ratio of 1:3 (w/w). The pan was hermetically sealed and allowed to equilibrate for 1 h before analysis. The sample pans were then heated from 20 to 100 °C at a rate of 5 °C/min. An empty pan was used as a reference.

2.8. Statistical analysis

Results are expressed as means ± one standard deviation. Subsequently, an analysis of variance (ANOVA) with Tukey's test was used to determine significant differences among the means at a significance level of $p < 0.05$. The data were subjected to correlation analysis and Pearson correlation coefficients were calculated by IBM SPSS Statistics (Version 22) Software version 13 (Minitab Inc., State College, PA).

3. Results and discussion

3.1. Texture profile analysis-textural characteristics

The textural parameters of the Yangyu jiaotuan like hardness, springiness, cohesiveness, gumminess, chewiness, and resilience determined by texture profile analysis (TPA) are presented in Figure 1. The TPA test mimics the human chewing action by subjecting a Yangyu jiaotuan to a compressive deformation followed by a relaxation and then a second compressive deformation [17]. The values of hardness, gumminess, chewiness, and resilience increased with the addition of both PPI and SPI in the Yangyu jiaotuan, and the values increased with the increasing of levels of addition. The changes of those parameters may be attributed to the low moisture content in Yangyu jiaotuan made with the addition of PPI and SPI, the moisture content of Nadine potato tuber was 89.31%±0.17, while the values for PPI and SPI were 5.44%±0.23 and 6.45%±0.39, respectively.

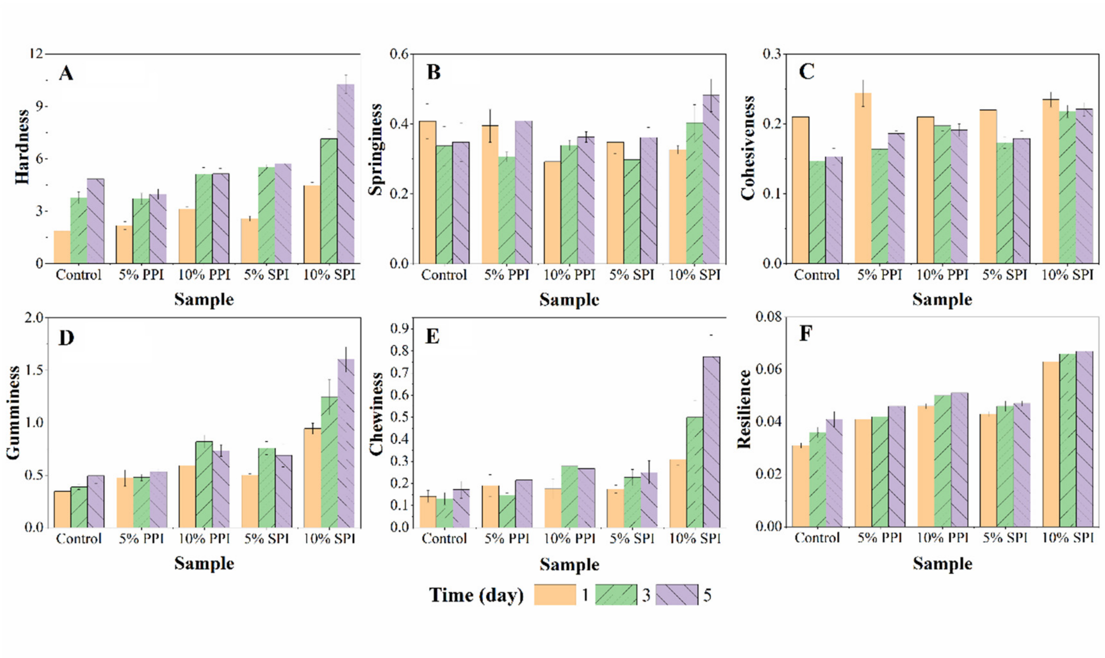

Figure 1. Effect of refrigerated storage on the textural parameters of Yangyu jiaotuan. A, hardness; B, springiness; C, cohesiveness; D, gumminess; E, chewiness; F, Resiliene.

Springinginess (Figure 2B) values varied from 0.29 for Yangyu jiaotuan made with 10% PPI to 0.48 for Yangyu jiaotuan made with 10% SPI, while the cohesiveness (Figure 2C) decreased with the addition of both PPI and SPI. The results showed that several important textural properties such as hardness and chewiness did not change significantly during the refrigerated storage. The extent of change in the texture of Yangyu jiaotuan was very small in samples containing PPI, reflecting its suitability for use in this product.

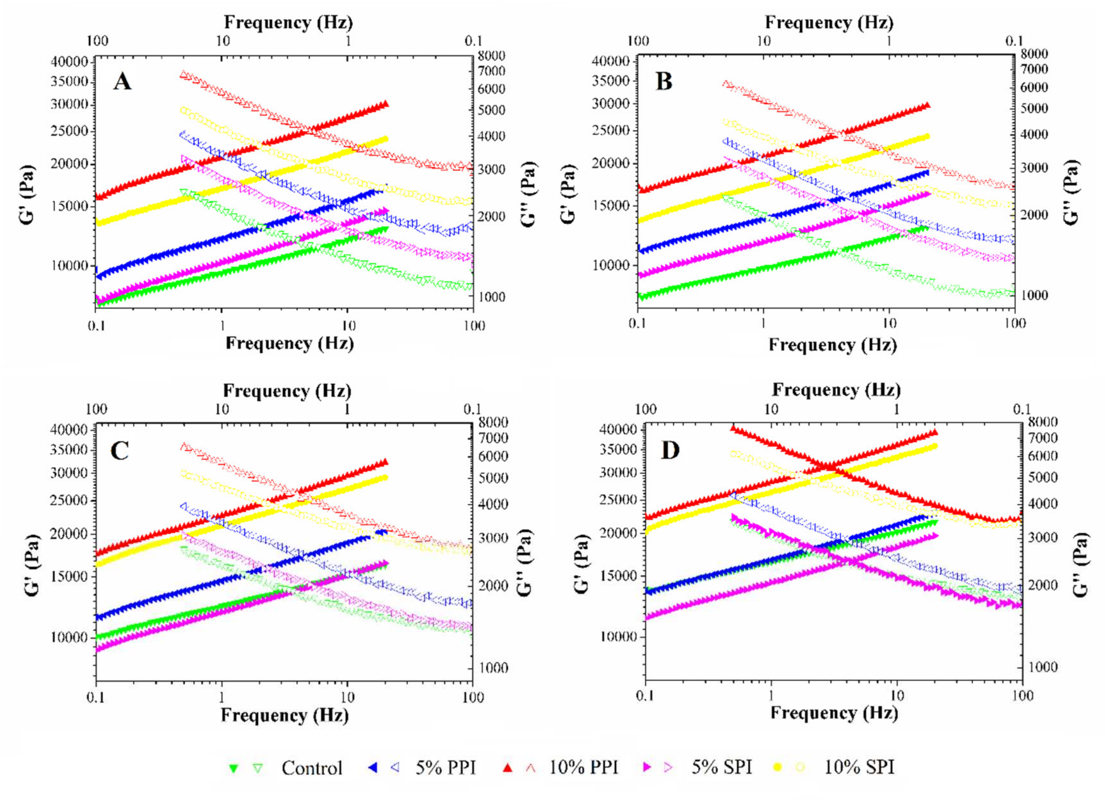

Figure 2. Effect of refrigerated storage on the dynamic rheological properties storage modulus (G′), and loss modulus (G″) of Yangyu jiaotuan. A, fresh prepared; B, 1-day of refrigerated storage; C, 3-days of refrigerated storage; D, 5-days of refrigerated storage.

3.2. Rheological properties

The dynamic rheological properties storage modulus (*G*′) and loss modulus (*G*″) are presented as a function of frequency in Figure 2 and Table 1, there were significant differences among the five mashed potato samples (with and without protein addition) with respect to frequency dependence during rheological measurements. In each case, the modulus versus frequency curves for the different samples exhibited no cross-over within the range of frequency accessed (Figure 2), *G*′, *G*″ and complex (*G**) increased while complex viscosity (η^*) and dynamic viscosity ($\acute{\eta}$) decreased with increasing frequency. All the values of rheological parameters *G*′, *G*″, *G**, loss tangent (tan δ), η^* and $\acute{\eta}$ increased with the addition of both PPI and SPI in the Yangyu jiaotuan, and the values increased with the increasing of levels of addition except the loss tangent.

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Table 1. Rheological properties of Yangyu jiaotuan.

Sample	Time (day)	Rheological properties during frequency sweep[a]					
		G'(Pa)	G''(Pa)	G^* (Pa)	tan δ	η^* (Pa s)	$\acute{\eta}$ (Pa s)
Control	0	12406.67±601.86a	2451.33±98.35a	12646.67±608.80a	0.20±0.00hi	100.650±4.84a	19.50±0.78a
	1	12670.00±357.63a	2263.33±64.47a	12870.00±361.66a	0.18±0.00d	102.423±2.88a	18.04±0.48a
	3	17130.00±742.23cd	2809.67±75.58b	17353.33±744.47c	0.16±0.008b	138.100±5.93c	22.36±0.60b
	5	21993.33±349.33f	3518.33±57.93de	22273.33±349.33e	0.16±0.00a	177.233±2.80e	28.00±0.47de
5% PPI	0	15983.33±1485.98c	3800.33±305.35e	16426.67±1514.48c	0.24±0.00m	130.733±12.04c	30.24±2.43e
	1	18786.67±85.05de	3782.67±4.73e	19160.00±80.00d	0.20±0.00i	152.467±.65d	30.10±0.04e
	3	19720.00±713.58e	3797.00±139.93e	20056.67±685.30d	0.19±0.00g	159.833±5.75d	30.22±1.12e
	5	23183.33±242.14fg	4339.33±65.455f	23583.33±250.07ef	0.19±0.00ef	187.667±1.95ef	34.53±0.52f
10% PPI	0	29863.33±814.51hi	6837.67±172.93j	30636.67±829.84gh	0.23±0.00l	243.800±6.56gh	54.42±1.38j
	1	28710.00±814.13h	5971.00±152.97h	29326.67±825.67g	0.21±0.00j	233.367±6.61g	47.52±1.22h
	3	31116.67±1001.67i	6269.67±190.08i	31740.00±1024.31h	0.20±0.00i	252.567±8.15h	49.90±1.51i
	5	38660.00±606.05j	7502.67±104.01k	39383.33±611.58i	0.19±0.00gh	313.400±4.8816i	59.70±0.83k
5% SPI	0	14190.00±1212.97b	3191.67±245.90c	14543.33±1241.02b	0.23±0.00k	115.767±9.86b	25.40±1.96c
	1	15786.67±440.72c	3148.67±59.69c	16100.00±446.43c	0.20±0.00i	128.100±3.59c	25.06±0.48c
	3	16466.67±775.13c	3106.33±125.60c	16756.67±785.13c	0.19±0.00f	133.367±6.25c	24.72±1.00c
	5	18473.33±1115.05de	3387.67±148.08cd	18783.33±1124.47d	0.18±0.00e	149.467±8.92d	26.96±1.18cd
10% SPI	0	24560.00±919.95g	5169.67±202.44g	25103.33±944.79f	0.21±0.00j	199.733±7.4782f	41.14±1.61g
	1	24710.00±547.81g	4554.67±69.21f	25126.67±553.38f	0.18±0.00ef	199.967±4.36f	36.24±0.55f
	3	29616.67±368.56hi	5263.00±48.54g	30080.00±370.41gh	0.18±0.00d	239.367±2.97gh	41.88±0.39g
	5	37663.33±1652.58j	6416.67±276.31i	38210.00±1677.71i	0.17±0.00c	304.033±13.32i	51.06±2.20i

Values with the same superscript in a column did not differ significantly ($p<0.05$). (G') = storage modulus, (G'') = loss modulus, (G^*) = complex modulus, (tan δ) = loss tangent, (η^*) = complex viscosity and ($\acute{\eta}$) = dynamic viscosity. [a] Rheological property values at 20 °C and 20 Hz.

The G' of control on the zero-day was about 12,000 Pa, remained the same on the first day, then increased to about 17,000 Pa on the third day, and increased to about 22,000 Pa on the 5th day, which indicates the Yangyu jiaotuan became hardened due to starch retrogradation during the storage. The values of Yangyu jiaotuan made with 5% plant protein (both PPI and SPI) are close to the control at

 doi:10.20944/preprints202305.0707.v1

zero-day, and during the storage of 1 day, 3 days, and 5 days, it has very similar characteristics to the control. At the end of even the 5th day, the Yangyu jiaotuan made with 5% SPI addition had the lowest viscosity values of among all, it was even lower than the control which indicates the palatability wise, the characteristics wise. The values of rheological parameters increased significantly when 5%PPI, 10%PPI, or 10% SPI was added, Yangyu jiaotuan made with 10% PPI gave the highest viscosity (all rheological parameters) in combination with potato, it was very different from the control, the formulation has very strong gel already.

Rheological techniques can also be used to monitor the development of viscoelastic properties of starch during retrogradation [18]. In this study, during the refrigerated storage at 4 °C, the values of G', G^*, and η^* increased significantly with the prolonging of storage time from 0 day to 5 days, while the values of G'', and $\acute{\eta}$ did not change significantly. The values of tan δ decreased, exhibited behavior contrary to G', G'', G^*, η^*, and $\acute{\eta}$, indicating the formation of stronger network structures in retrograded pastes.

Table 2 illustrates the extent of change in G', G'', and η^* of percentage. For the control sample, the value of G' after 5 days has increased 77.27%, while it was lower for the sample containing 5% PPI, it was 45% increase. The extent of change in G'' and $\acute{\eta}$ in terms of percentage show absolutely the same pattern as the G' values, this means that protein has helped the sample to prevent the retrogradation because it didn't harden. Usually, consumers like to eat the soft, so we can keep Yangyu jiaotuan soft during the storage period by adding plant proteins. Moreover, the value of G' after five days has increased 29.46% and 53.35% for the Yangyu jiaotuan containing 10% PPI and 10% SPI, which indicate that with a high extra protein addition, PPI is more effective in preventing the starch retrogradation and ultimately obtaining more smooth, consistency or a soft consistency over the storage period.

Table 2. The extent of change in G', G'', and $\acute{\eta}$ in terms of percentage.

Sample	Time (day)	G' change (%)	G'' change (%)	η^* change (%)
Control	1	2.12	-7.67	1.76
	3	38.07	14.62	37.21
	5	77.27	43.53	76.09
5% PPI	1	17.54	-0.46	16.62
	3	23.38	-0.09	22.26
	5	45.05	14.18	43.55
10% PPI	1	-3.86	-12.67	-4.28
	3	4.20	-8.31	3.60
	5	29.46	9.73	28.55
5% SPI	1	11.25	-1.35	10.65
	3	16.04	-2.67	15.20
	5	30.19	6.14	29.11
10% SPI	1	0.61	-11.90	0.12
	3	20.59	1.81	19.84
	5	53.35	24.12	52.22

Although, the rheological parameters have already giving a lot of information, a lot about different proteins, and how they are affecting starch retrogradation. But to find out the mechanism by which these proteins are affecting, because that'll make it more interesting, rather than leaving it on a superficial, and how we can make use of that. Whether there is any molecule level interaction among them in terms of the bonding, or they're just helping to absorb the water which comes out of starch due to syneresis. What is the mechanism? It worth further study.

The Pearson correlation coefficients for the relationships between textural and rheological properties are presented in Table 3. Hardness, Gumminess, Chewiness and Resilience showed a positive correlation with G' ($r = 0.658, 0.680, 0.659, 0.692$, respectively), whereas rheological properties

 doi:10.20944/preprints202305.0707.v1

such as G0, G00 and peak viscosity showed a positive correlations (r = 0.690, 0.847, 0.736, respectively).

Table 3. Pearson correlation coefficients for various properties of textural and rheological properties.

	Hardness	**Springiness**	**Cohesiveness**	**Gumminess**	**Chewiness**	**Resilience**	*G′*	*G″*	*G**	**tan *δ***	*η**	*ή*
Hardness	1.000											
Springiness	0.203	1.000										
Cohesiveness	0.026	0.318*	1.000									
Gumminess	0.888**	0.217	0.429**	1.000								
Chewiness	0.876**	0.447**	0.448**	0.964**	1.000							
Resilience	0.795**	0.146	0.431**	0.938**	0.871**	1.000						
G′	0.658**	0.144	0.206	0.680**	0.659**	0.692**	1.000					
G″	0.518**	0.070	0.249	0.578**	0.539**	0.619**	0.973**	1.000				
*G**	0.654**	0.141	0.208	0.677**	0.655**	0.690**	1.000**	0.975**	1.000			
tan *δ*	-0.393**	-0.214	0.368*	-0.186	-0.252	-0.069	0.078	0.296*	0.085	1.000		
*η**	0.654**	0.141	0.208	0.677**	0.655**	0.690**	1.000**	0.975**	1.000**	0.085	1.000	
ή	0.517**	0.070	0.249	0.578**	0.539**	0.619**	0.973**	1.000**	0.975**	0.296	0.975**	1.000

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

The correlation between these texture parameters and *G″* and *G** shows the same pattern, because gumminess, chewiness, and resilience showed a strong positive correlation with hardness, while *G″*, *G**, *η**, and *ή* also has a strong positive correlation with *G′*.

3.3. Starch hydrolysis (%) and estimated glycaemic index

The starch hydrolysis (%) of Yangyu jiaotuan during in vitro oral-gastro-small intestinal digestion is shown in Figure 3. During the simulated oral digestion phase (O and G0), α-Amylase is generally well integrated within the food bolus and continues to release some glucose, starch hydrolysis (%) observed during the oral digestion process ranged from 2.27% to 14.60%. During the simulated gastric digestion phase (G0 and G30), the starch hydrolysis (%) of all samples were barely changed indicating the activity of α-Amylase is very low due to the extremely low pH of 2 (Figure 3). During the simulated small intestinal digestion, the starch hydrolysis (%) of all samples increased rapidly, especially at the initial phase. From Figure 3, it was obvious that the starch hydrolysis (%) of all samples decreased with the extension of refrigerated storage time from 0 to the 5th day, the gradually decreasing starch hydrolysis (%) of the 0-day (76.34%), 1-day (72.17%), 3-day (65.09%) and 5-day (57.19%) of the plain Yangyu jiaotuan (control) at the I15 point indicated that refrigerated storage resulted in the starch retrogradation process and subsequent the rate of enzymatic digestion, which is consistent with our previous study [14].

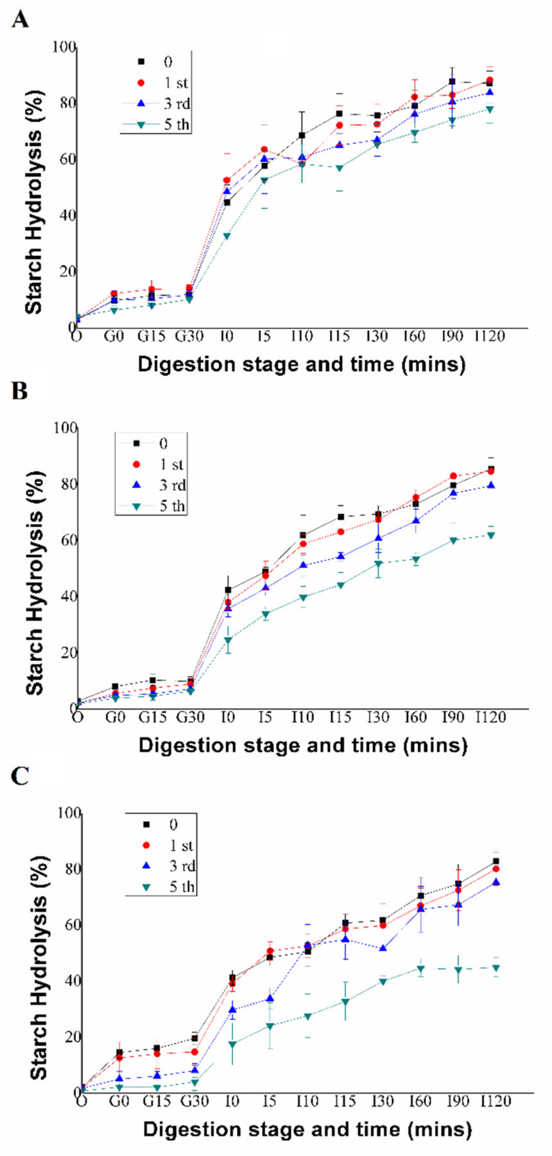

Figure 3. Starch hydrolysis (%) of plain Yangyu jiaotuan (A), mixed with 5% PPI (B), and 10% PPI (C) after refrigerated storage at 4 °C for 0, 1, 3 and 5 days. SEM images of washed Yangyu jiaotuan.

Equilibrium starch hydrolysis percentage (C_∞), areas under hydrolysis curves (AUC), hydrolysis index (HI), and estimated glycaemic index (*e*GI) were estimated by fitting a first-order equation model according to the reported method [16]. The HI of fresh prepared plain Yangyu jiaotuan was 129.84 while the white read was used as a reference. The HI and the *e*GI of all the samples decreased with increasing refrigerator storage time indicating storage affected the rate of enzymatic digestion (Table 4). The experimental C_∞ of 5% PPI and 10% PPI samples after 5-days refrigerated storage were significantly lower than the values of other samples, while the differences of values of other samples are not significant. For the same refrigerated storage time of 0, 1, 3, and 5 days, the higher the amount of protein added, the lower the HI and *e*GI values. The 10% PPI after 5-days of refrigerator storage had the lowest HI (41.76) and *e*GI (62.63) values, which is significantly lower than the other values of the control and 5% PPI. Factors that influence the kinetics of starch digestion are the nature of starch, physical form, protein and lipids interactions, presence of antinutrients enzyme inhibitors, and food processing [19].

 doi:10.20944/preprints202305.0707.v1

Table 4. Kinetics of starch hydrolysis percentage, hydrolysis index (HI) and estimated glycaemic index (*e*GI) of plain Yangyu jiaotuan (control), with 5% pea protein isolate (5% PPI) and with 10% pea protein isolate (10% PPI).

Sample	Time (day)	$C_{\infty\ experimental}$ (%)	$C_{\infty\ estimated}$ (%)	AUC	HI	*e*GI
Control	0	87.13±4.39c	88.31±3.83de	9728.93±164.21e	129.84±2.19e	110.99±1.20e
	1st	88.33±4.79c	87.06±1.30de	9618.57±288.71e	128.37±3.85e	110.18±2.12e
	3rd	83.83±3.89c	83.14±6.40cde	9137.26±411.84de	121.95±5.50de	106.66±3.02de
	5th	78.11±5.13c	75.46±5.11cd	8418.05±682.57cde	112.35±9.11cde	101.39±5.00cde
5% PPI	0	85.59±3.97c	82.35±3.68cde	9070.40±394.32de	121.05±5.26de	106.17±2.89de
	1st	84.66±2.45c	81.07±2.06cde	8922.15±222.79de	119.08±2.97de	105.08±1.63de
	3rd	83.33±0.74c	79.61±3.19cde	8679.75±741.24cde	115.84±9.89cde	103.31±5.43cde
	5th	62.09±2.97b	59.14±3.44b	6478.45±512.51b	86.46±6.84b	87.18±3.76b
10% PPI	0	82.86±7.47c	79.01±5.82cde	8534.99±598.30cde	113.91±7.98cde	102.25±4.38cde
	1st	80.18±5.92c	76.60±4.70cde	8257.78±602.94cd	110.21±8.05cd	100.21±4.42cd
	3rd	75.41±5.90c	70.70±4.91c	7501.22±507.75c	100.11±3.72c	94.67±3.72c
	5th	45.06±3.43a	43.06±3.28a	4822.26±405.86a	64.36±5.41a	75.04±2.97a

Figure 3 and Table 4 clearly show that the newly developed product with extra plant protein has lower starch hydrolysis or estimated glycaemic index than the control sample implying the gel network made with globular protein granules could act as a physical barrier impeding amylase accessibility to the encapsulated starch, in a similar manner to that seen with cell walls. The protein gel network appeared to hinder the free access of amylolytic enzymes to the encapsulated starch, therefore providing a slow and extended-release of glucose during *in vitro* digestion. This means the high protein Yangyu jiaotuan are disassembled and release nutrients in the digestive tract at a slower rate with greater satiety as opposed to plain mashed potato.

The ultimate vision of this study is not to make a replica of natural foods, but rather to be inspired by structure-functionality linkages encoded in them, to enable the design of nature-like food systems with similar or enhanced functionality. We develop a category of BPF product of high protein Yangyu jiaotuan that can be tailored to allow their uses in specific health-promoting applications to help prevent or combat chronic diseases include lowered caloric content and prolonged satiety for obesity management, slow and sustained release of glucose in the small intestine and delivery of resistant starch to the large intestine for decreased risks of diabetes and improved colonic health.

3.4. Microstructural

Microphotographs of Yangyu jiaotuan are shown in Figure 4. As shown by the microphotographs of a light microscope (Figure 4A, B, and C), large size particulars in blue and small size particulars in golden can be distinguished, they refer to starch (stained by 1% iodine indicator) and globular protein, respectively. A fairly amount of golden granules can be found in the Yangyu

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jiaotuan made with 5% PPI (Figure 4B) and 5% SPI (Figure 4C), the morphologies characteristics such as color and shape were very similar, but the particle size of SPI was relatively higher than that of PPI.

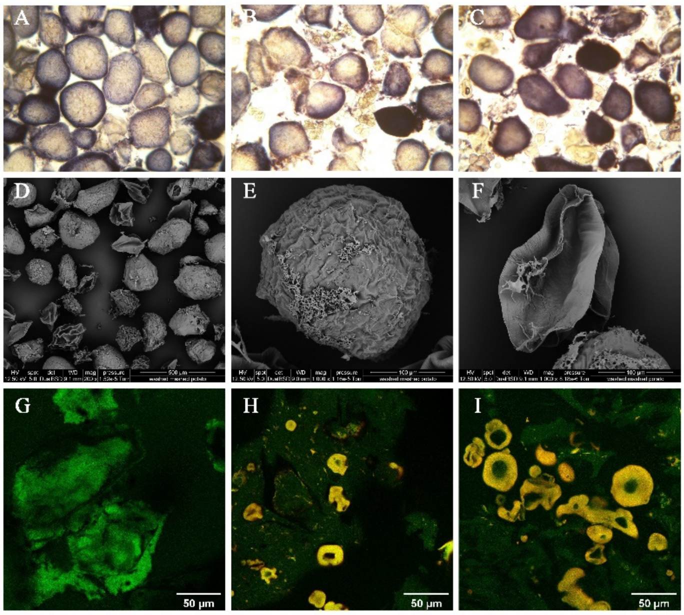

Figure 4. Light micrographs (100×) of Yangyu Jiaotuan (A, control; B, with 5% SPI; C, with 5% PPI), SEM images of washed Yangyu jiaotuan (D, 200×; E, and F, 1000×), the CLSM time-lapse images of fresh prepared Yangyu jiaotuan stained by fast green and FITC (A, plain Yangyu jiaotuan; B, with 5% PPI; C, with 10% PPI).

After the addition of extra plant protein, two different types of gel networks were formed: fine-stranded and coarse networks [12]. The absence of adhesion between starch matrix should be disregarded as they are not features of the different samples but a problem of sample preparation. A large amount of liquid was introduced during the staining of the light microscope. A strong gel network of the starch matrix was formed in Yangyu jiaotuan, which can be confirmed by the results of the CLSM images (Figure 4G, H, and I). The opaque coarse gels network defined as the white aggregate was made with starch matrix, Yangyu jiaotuan with and without extra plant protein addition consist mainly of a continuous phase (amylose/amylopectin matrix) due to the disruption and complete solubilization of the potato starch granules by heating.

Another opaque fine-stranded gel network was particulate gel made with globular protein granules [20]. In both PPI (Figure 4C) and SPI (Figure 4D) Yangyu jiaotuan, a protein network structure can be seen composed of protein aggregate clumps and small protein clusters, which are distinguishable from the starch matrix. Tseng et al. also observed that SPI gels exhibited a particulate porous network structure [21], Alvarez et al. reported two different types of gel network in mashed potatoes enriched with SPI [12].

To achieve a better understanding of the starch matrix of Yangyu jiaotuan, fresh prepared Yangyu jiaotuan was washed with plenty of water and followed by freeze-drying, the microstructure of the systems was studied by SEM (Figure 4D, E, and F). Individual cells were successfully isolated and captured, the size of freeze-dried potato cells ranged between 100 to 200 microns, which is much smaller than the potato parenchyma cells ranged between 100 to 1000 microns [11]. The starch matrix is tightly packed within the cell wall polymer network, and the folds on the surface of microspheres can explain the dehydrating effect of freeze-drying on the shrinking cell volume. Micrographs also revealed the presence of cell wall cementing materials as well as cell fr aments are embedded in the continuous solubilized starchy matrix (Figure 4D and F), which was consistent with the previous report [12]. The native starch granules absorbed abundant cell fluid and gelatinized during

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hydrothermal cooking, followed by the separation of intact cells (instead of rupturing) during Yangyu jiaotuan processing due to the drastic mechanical shearing. Once there has been a substantial level of cell separation, it becomes rather difficult to fracture individual cells by any mechanical means [22]. Berg, Singh, Hardacre et al. and Tydeman et al. also reported that hydrothermal processing of plant foods (e.g. legumes and carrots) and subsequent mechanical shear applied within the resulting puree induce separation of intact cells without breaking them open [23,24].

During the simulated oral digestion and simulated gastric-small intestinal digestion, microstructural changes of mashed potato, mixed with pea protein were also revealed by SEM (**Figure 5**). After 2 minutes of oral digestion (Figure 5A), the morphology of the starchy matrix did not change compared with that of freshly prepared Yangyu jiaotuan (Figure 4D and E). Similarly, there was no obvious change after 30 minutes of simulated stomach digestion (Figure 5B), this observation was attributed to the absence of amylases in the gastric juice with the minimal hydrolysis observed being attributed to acid pH [25]. During the initial phase of simulating small intestine digestion, the starchy matrix shrank to become smaller and the cell wall polymer network increased (Figure 5C). After 10 minutes of simulating small intestine digestion, a large number of cell wall polymer network fragments were reminded (Figure 5D). As the digestion process progresses, the starch matrixes are nearly invisible, leaving only the cell wall polymer network fragments and the pea protein particles (Figure 5E), *in vitro* starch digestion assays combined with SEM techniques demonstrated that gelatinized starch, which is tightly packed within biopolymer matrices in cooked starch matrixes, was enzyme-digested in a layer-by-layer fashion (Figure 5C2), this means that digestion progressed from the outer towards the center of the starch-entrapped microspheres [1]. Figure 5F is a plain mashed potato without additional plant protein, no starch matrix could be found after 120 minutes of simulated small intestine digestion, and all that remained were cell wall polymer networks that could not be digested by amylase.

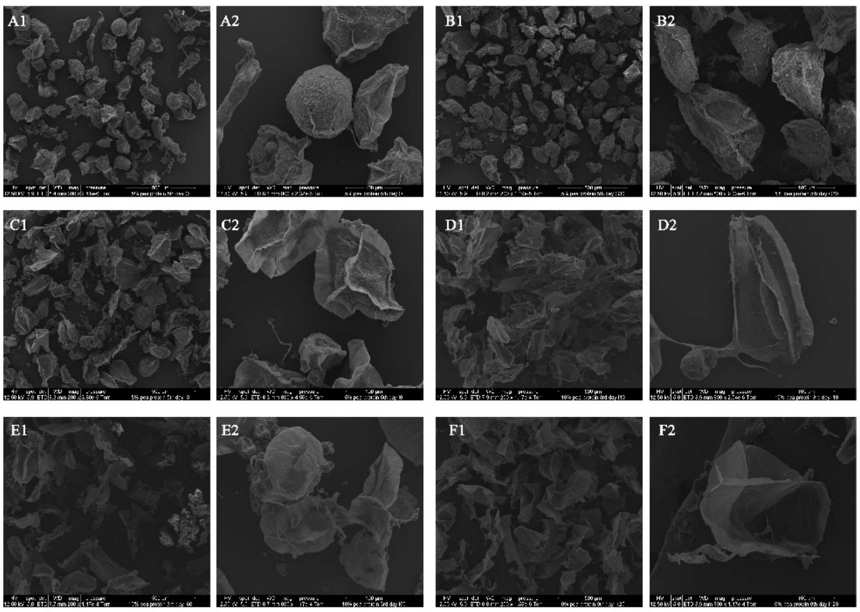

Figure 5. SEM images of Yangyu jiaotuan digesta with 5% pea protein after 5-day refrigerated storage at O2 (A), 5% pea protein after 5-day refrigerated storage at G30 (B), 5% pea protein after 5-day refrigerated storage at I0 (C), 10% pea protein after 3-day refrigerated storage at I10 (D), 10% pea protein after 3-day refrigerated storage at I60 (E), and fresh prepared plain mashed potato at I120 (F).

3.5. Particle size distribution and thermal properties of Yangyu jiaotuan digesta

The particle size distribution for Yangyu jiaotuan digesta with 5% PPI after 3-days of refrigerated storage are shown in Figure 6. The bimodal peaks of the particle size distribution curves show that the newly developed Yangyu jiaotuan contains two different sizes of particles, the smaller particles corresponded to PPI (~30 μm), which is consistent with the CLSM and SEM results (Figure 4I and

Figure 5E2). Our previous study revealed that plain cooked potato tuber after simulated digestion also contains some small particles sized between 15.2-35.1 μm [14], which indicated that the small particles in the Yangyu jiaotuan also contain some starch granules [26], which can be confirmed by the volume of large particles represented in Yangyu jiaotuan (~10%) was much greater than that of 0.5–1.5% represented in plain cooked potato. The larger particles in Yangyu jiaotuan corresponded to the fragments of a gel network of the starch matrix [26], which is consistent with the SEM results (Figure 5). The results also show that the integrated volume of the small particle sizes increased and the volume of the large particle sizes decreased as the digestion time extended, indicating that the fragments of starch matrix became smaller and smaller during the prolonged digestion.

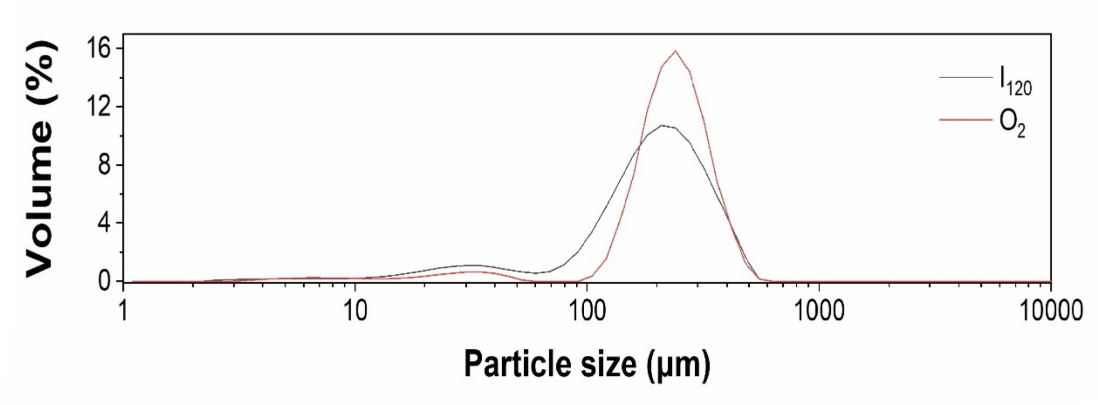

Figure 6. Particle size distribution for Yangyu jiaotuan digesta with 5% PPI after 3-days of refrigerated storage.

The results about differential scanning calorimetry (DSC) thermogram of Yangyu jiaotuan digesta samples during the simulated oral-gastric-small intestinal digestion were showed in Figure 7. The peak temperature (T_P) of O2 and G30 were found to be 75.47 and 75.75 °C respectively, the endothermic peaks reduced in samples as the simulated digestion time extended, the samples of I10, I10, and I120 did not show endothermic peaks, indicating that the crystalline region was gradually destroyed [27]. Besides, starch is quickly digested in the simulated small intestine, in particular the I120 sample, there is no starch matrix residue. These results were inconsistent with those of starch hydrolysis (Figure 3) and SEM (Figure 5).

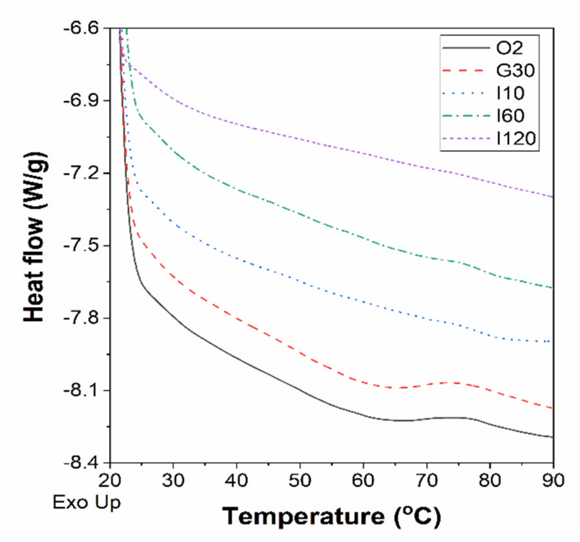

Figure 7. Differential scanning calorimetry (DSC) thermogram of Yangyu jiaotuan digesta with 5% PPI after 5-days of refrigerated storage during the simulated oral-gastric-small intestinal digestion.

4. Conclusions

The design and manufacturing of high protein-low glycaemic biomimetic potato food Yangyu have broad commercialization, the prospects are very exciting. Although potato tubers already contain high-quality protein, adding extra plant proteins (soy protein isolate-SPI and pea protein isolate-PPI) as nutrient enhancers to the Yangyu jiaotuan will have many benefits: Firstly, the glycemic index can be reduced; Secondly, the protein content can be increased; Thirdly, the product can maintain the same taste and texture; Fourthly, the price is affordable; Fifthly, it belongs to the convenience food; Finally, such a potato product is a staple food with the consumer base.

Based on the popularity of this potato-based product in China, this product was selected to create a high protein and low glycaemic version of Yangyu Jiaotuan. This project can be very useful for the potato processing industry of China if it decides to manufacture this new Yangyu Jiaotuan, which is a very healthy and nutritious food especially for people with diabetes and obesity. The starch hydrolysis during in vitro gastro-small intestinal digestion clearly shows that the newly developed product has lower starch hydrolysis or estimated glycaemic index than the control sample. It provides more protein and a lower glycaemic index and the convenience that fit the modern lifestyle and the demands of the millennial population in China. We expect that it will be a big success if this product comes to the restaurant market in China. This product also fits with the China Government's policy of "Potato as a staple food" and complies with the biomimetic potato food very well.

Acknowledgments: This work was supported by the Special Fund Project for High-Tech Industrialization of Science and Technology Cooperation Between Jilin Province and Chinese Academy of Sciences (Grant No. 2021SYHZ0005), China Agriculture Research System (Grant No. CARS-9), Key Research and Development Plan of Ningxia Hui Autonomous Region (Grant No. 2020BBF03018), and the Government-sponsored study abroad Program of the Chinese Academy of Sciences (Liufangxijizi [2018] No. 35). The authors would also like to thank the SFAT and Ridder CoRE for hosting Fan-kui Zeng and funding the study.

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