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Protein-starch matrix plays a key role in enzymic digestion of high-amylose wheat noodle

Hai-Teng Li[a,b], Zaifen Li[a], Glen P. Fox[c], Michael J. Gidley[a], Sushil Dhital[a,d,*]

[a] The University of Queensland, Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, Brisbane, QLD 4072, Australia
[b] School of Food and Biological Engineering, Jiangsu University, Zhenjiang, Jiangsu Province 212013, China
[c] Department of Food Science and Technology, 2158 Robert Mondavi Institute, University of California Davis, CA 95616, USA
[d] Department of Chemical Engineering, Monash University, Clayton, VIC 3800, Australia

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ABSTRACT

Wheat flour, consisting of a complex matrix of starch and protein, is used as a representative model of whole food here to investigate the binary interaction in relation to amylose level and hydrothermal treatment in noodles as a food exemplar. Noodle made of high-amylose wheat (HAW) flour showed an eight-fold higher resistant starch content, compared to the wild type. Protein removal under simulated intestinal digestion conditions resulted in higher starch digestion rate coefficients in raw and cooked flours. In cooked flours, the substrate becomes similarly accessible to digestive enzymes regardless of protein removal. The results indicate that the increased protein content in native HAW flour and thermal stability of starch in HAW noodles lead to higher food integrity and consequently enhance the resistance against α-amylase digestion. Overall, the study suggests that a diversity of starch-protein interactions in wheat-based food products underlies the nutritional value of natural whole foods.

1. Introduction

Starch is a major component in cereals, located among other macronutrients including protein. The interaction of starch with protein has been reported to have impacts on food quality and functionality, *e.g.*, noodle quality (Konik, Miskelly, & Gras, 1993), malting quality (Brennan, Harris, Smith, & Shewry, 1996), bread loaf volume (He & Hoseney, 1992), starch pasting (Debet & Gidley, 2006; Hamaker & Griffin, 1993; Zhang & Hamaker, 2003), rate of starch digestion both *in vitro* (Bhattarai, Dhital, & Gidley, 2016; Zhang & Hamaker, 1998; Zou, Sissons, Gidley, Gilbert, & Warren, 2015) and *in vivo* (Berti, Riso, Monti, & Porrini, 2004; Jenkins et al., 1987), as reviewed recently (Dhital, Brennan, & Gidley, 2019). The consumption of natural foods containing complex matrices could be more nutritious and healthier than those with isolated nutrients (Fardet & Rock, 2014). Starch that is slowly digested or resistant to digestion in the human gastrointestinal tract is known to have beneficial nutritional outcomes. On the other hand, rapid digestion of starch leads to a high glycemic response, which is considered as a risk factor for type 2 diabetes. However, at least partially due to the complexity of food structure, it is difficult to clarify or quantify the contribution of macronutrient interactions to starch digestibility in a real food environment.

As a model system, digestion of each of the three macronutrients in raw wheat flour (starch, protein, lipid) was shown to be influenced by interactions with each of the other two macronutrients (Bhattarai et al., 2016). Protein is the second abundant macronutrient in wheat. A continuous proteinaceous matrix surrounding starch granules can be formed after water hydration of wheat flours, *e.g.* during dough formation, which is an essential intermediate step to transform flours into many products (*e.g.*, bread, pasta, and noodle). However, there is still a lack of information about macronutrient interactions in whole food after the exposure to hydrothermal processing which is more relevant to the food forms usually consumed. Thus, the approach proposed here to study the effect of macronutrient interactions on starch digestibility in wheat-based foods is to: (1) identify food microstructures with distinct starch digestibility in a wheat-based food product; (2) prepare representative materials of the food structures, using wheat flour as a model whole food; (3) investigate starch digestibility of representative materials in relation to the presence of other macronutrients.

Although using model materials inevitably ignores some characteristics of large-scale food structures (*e.g.*, texture properties related to food processing, and starch forms with various extent of gelatinization), combinations of components can be used to approximate the complexity of macronutrient interactions in foods. A similar logic has

* Corresponding author at: Department of Chemical Engineering, Monash University, Clayton, VIC 3800, Australia.
E-mail addresses: haiteng.li@uq.net.au (H.-T. Li), sushil.dhital@monash.edu (S. Dhital).

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been applied in previous studies on cell wall-starch interactions using isolated individual cells. These studies explicitly revealed the barrier properties of cell walls on starch digestion (Bhattarai, Dhital, Mense, Gidley, & Shi, 2018; Dhital, Bhattarai, Gorham, & Gidley, 2016; Ding et al., 2019; Li, Gidley, & Dhital, 2019b).

High-amylose starches have reduced digestion rate and a higher level of resistant starch (as a type of dietary fibre) compared with regular starches, providing opportunities for incorporation into food products (*e.g.*, noodle) and diets for better nutritional outcomes (Li, Gidley, & Dhital, 2019a; Newberry et al., 2018). Noodle is a popular staple food around the world, and wheat flours in Asia are widely consumed in form of noodles; replacing regular wheat flour with HAW flour in noodle could potentially improve fibre intake in broad populations. Compared to wild type, HAW with apparent amylose content up to 93% showed distinct molecular and microstructural features of starch as characterized previously (Li, Dhital, Slade, et al., 2019); the differences could induce structural changes of protein-starch matrix in HAW foods, and lead to different food properties. It is hypothesized that the protein-starch matrix in HAW is a factor that contributes to the changes of noodle properties and slower starch digestion, compared to wild type.

In this study, noodle as a food exemplar was made using wheat flours with regular and high amylose contents, and analysed in terms of food texture, *in vitro* digestibility and morphology to identify the food structures responsible for digestion performance. Two forms of wheat flours (raw and hydrothermally treated) were used to model the food structures in noodle and investigate the effect of starch-protein interactions on starch digestibility. The diversity of protein-starch interactions in relation to cooking process and amylose level and the mechanism of how these interactions limit starch digestion will be discussed.

2. Materials and methods

2.1. Materials

High-amylose wheat flours were obtained and supplied by a breeding program in Arcadia Bioscience (CA, US). The wild-type wheat line (RS01) was used as reference controls for the mutants (RS137 and RS100), with amylose contents of 37, 47, and 93%, respectively. The high-amylose wheat used for noodle making was RS100 harvested in 2017. Mature grains were milled into flours at the California Wheat Commission (Woodland, CA) using a Brabender Quadramatic Senior mill. The protein content was determined using the Kjeldahl method, with a nitrogen to protein conversion factor of 5.7. Total starch content (%, dry basis) was measured by a Total Starch Assay Kit (Megazyme, Ireland). The composition of the samples is shown in Table S1.

2.2. Noodle quality analysis

Yellow alkaline noodles (YAN) were prepared at the Queensland Department of Agriculture and Fisheries Leslie Research Centre (Toowoomba, QLD, Australia) using a standard method for commercial wheat quality testing. Briefly, wheat flour was mixed with an alkaline lye solution (containing NaCl, K_2CO_3 and Na_2CO_3), followed by adjusting water content to 32–34% in a Hobart mixer. An Ohtake laboratory noodle machine (Ohtake, Japan) was used for sheeting after compression and resting (20 min, 25 °C).

Noodles were cooked in boiling water for 10 min, as described in AACC Approved Method 66–50. Noodle colour was determined by Chroma Meter CR-400 (Konica Minolta, Japan) after calibration according to the manufacturer's instructions. The parameter b* generated by the colorimeter indicates yellowness-blueness (positive value for yellow and negative value for blue). Noodle texture was analysed using a TA-XT plus (Stable Micro Systems Ltd., UK). Five strands of freshly cooked noodles were placed parallel on a flat metal plate and compressed twice to 70% of the noodle height at a speed of 10 mm/min. The height of the first peak was recorded as hardness or firmness. Springiness is the ratio between the recovered height after first compression and the height of the first compression. Cohesiveness is the ratio between the area under the second peak and the area under the first peak. Resistant starch was measured using a Resistant Starch Assay Kit (Megazyme, Ireland).

2.3. In vitro digestion of noodles

A standardized *in vitro* digestion method (Minekus et al., 2014) was performed with slight modifications. The digestion fluids of the simulated gastric and intestinal phases (referred to as SGF and SIF) were prepared and adjusted to reach pH values of 3.0 and 7.0, respectively. Raw noodles (100 mg) were cut into ~ 2 mm in size and weighed in a 50 mL falcon tube. The samples were cooked in 2 mL boiling water for 10 min, and then cooled for 5 min at room temperature. The "0h" aliquots were collected after 30-min digestion in 4 mL SGF containing pepsin (800 units, 1:2500LR, Chemsupply), but before the addition of 8 mL SIF containing α-amylase (50 units, Sigma A6255-100MG, from porcine pancreas). The definitions and assays of enzymatic activity are from Minekus et al. (2014). The digestion solution was incubated with a magnetic stirrer bar in a water bath (37 °C) with constant mixing (200 rpm). The aliquots (50 μL) were collected at a range of times and added to 450 μL Na_2CO_3 (0.5 M) solution to stop amylase hydrolysis, followed by centrifugation (2000 g, 10 min). The released reducing sugar in the supernatant was measured using the 4-hydroxybenzoic acid hydrazide (PAHBAH) assay. An aliquot of the supernatant (100 μL) was transferred into 1.0 mL freshly-prepared PAHBAH solution (9:1 mixture of 0.5 M sodium hydroxide and 5% (w/v) PAHBAH in 0.5 M hydrochloride), followed by incubation in a water bath (100 °C) for 5 min and then cooling to ambient temperature. The absorbance was measured at 410 nm and used for the calculation of the digested starch (%) according to Zou, Sissons, Warren, Gidley, and Gilbert (2016).

2.4. Wheat flour digestion

Wheat flour (as a model whole food) subjected to protein removal under stimulated digestion conditions was used to elucidate the effect of protein matrix in reducing starch digestion rates. The reductionist approach excludes the food structural variables (*e.g.*, noodle compactness) in relation to starch digestibility. Amylose content in wheat modulates starch functionality, *e.g.*, swelling ability (Li, Gidley, et al., 2019a); wheat lines with high amylose content were compared to wild type, in order to investigate if the changes in starch functionality result in differences of the binary interaction (*i.e.*, physical entrapment by protein matrix). Wheat flour (100 mg) was weighed in a 50 mL falcon tube. Cooked wheat flour was cooked in 2 mL boiling water for 10 min and cooled for 5 min at room temperature. Protein was digested with 4 mL SGF for 30 min, followed by adding 8 mL SIF containing α-chymotrypsin (Sigma C4129-1G, from bovine pancreas) and trypsin (Sigma T4799-5G, from porcine pancreas) for up to 6 h. The enzyme activities of α-chymotrypsin and trypsin in SIF were 25 unit/mL and 100 unit/mL respectively as described in the standardized method (Minekus et al., 2014). Aliquots (150 μL) were collected at a range of times and immediately mixed with 150 μL 24% trichloroacetic acid, followed by centrifugation at 2000 g for 5 min. Protein in the supernatant was analyzed using a Pierce BCA protein assay (Thermo Scientific kit 23225). Bovine serum albumin was used to prepare a standard curve.

The protein was pre-digested for 30 min (time in SGF) and 4 h (time in SIF), with the control treated with SGF buffer for 30 min and SIF buffer for 4 h without adding enzymes. Wheat flours after protein predigestion were further digested again in SIF for another 6 h to investigate starch digestibility by adding α-amylase (50 units). The aliquots (50 μL) were collected at a range of times. The data were analysed using a general approach to fitting starch digestion kinetics as described

in Li, Dhital, Gidley, and Gilbert (2019). The method includes a step to check the number of digestion steps. The uncertainty check of the fitted parameters suggested a single rate constant (k) was sufficient to fit the digestion curves of wheat flour materials after protein removal. Thus, starch digestion data have been fitted to a first-order equation: $C_t = C_\infty(1\text{-}e^{-kt})$, C_t: the percentage of starch digested at a given time (t), k: rate coefficient, C_∞: estimated percentage of starch digested at the end point of the reaction.

2.5. Confocal scanning laser microscopy (CSLM)

The morphology of protein/starch matrices (wheat flours and the inner and outer section of cooked noodles) was observed under an LSM 700 confocal microscope (Carl Zeiss, Germany) as described previously (Bhattarai et al., 2016). Fluorescamine (F9015, Sigma) and fluorescein isothiocyanate (FITC; F7250, Sigma) were used for labelling protein and starch, respectively.

2.6. Fourier transform infrared spectroscopy (FTIR)

An FTIR spectrometer (PerkinElmer Spectrum One) fitted with a PerkinElmer UATR single bounce ATR accessory with a diamond crystal was used to obtain IR spectra as described previously (Warren, Gidley, & Flanagan, 2016). Cooked samples of wheat flours and noodles were prepared as described above, while uncooked samples were treated with water at room temperature (~20 °C). All the samples were dried overnight in a vacuum oven and equilibrated in a desiccator containing a saturated K_2CO_3 solution at room temperature (~20 °C) to obtain similar moisture contents (~12% w/w). The samples were then ground into powder of size range 100–1000 μm using a coffee grinder as described elsewhere (Zou et al., 2015). The spectra were plotted after standard normal variate (SNV) normalization.

2.7. Statistical analysis

All experimental data are presented as mean ± SD for at least triplicate measurements. Significant differences at the $p < 0.05$ confidence level were analysed by Tukey's HSD pairwise comparisons in Minitab 17 (Minitab, Inc., State College, PA, US).

3. Results

3.1. High-amylose wheat noodle

HAW with very high amylose content (> 80%) was used here, while wheat flours used in the previous attempts to make higher-amylose wheat foods (Morita et al., 2002; Van Hung, Yamamori, & Morita, 2005) had no more than 38% amylose content. The quality of HAW noodle (YAN) in terms of appearance, texture and resistant starch was compared to WT noodle (Table 1). Yellowness, as an important colour parameter for YAN, was similar between the two noodles in the uncooked form, while HAW noodles showed a higher value of yellowness after cooking. The hardness of HAW noodle was higher, along with reduced springiness and cohesiveness, as compared to the control. HAW starch has limited swelling ability as measured by Rapid Visco Analyzer (Li, Dhital, Slade, et al., 2019), which could contribute to the higher hardness. Reduced swelling ability is generally associated with increasing amylose content. The differences in functionality between regular and HAW could either be an advantage or a disadvantage depending on what noodle qualities are desired (Li, Dhital, & Wei, 2017). For example, an increase in hardness due to limited starch swelling could be favourable for YAN, while highly swelling starch (low amylose starch) may be preferred for other widely consumed noodle types such as white salted noodle (WSN). Previous studies also reported that incorporation of high-amylose flour enhances noodle hardness (Baik & Lee, 2003; Guo, Jackson, Graybosch, & Parkhurst, 2003) and reduces noodle springiness (Kaur et al., 2016) and cohesiveness (Baik & Lee, 2003; Guo et al., 2003).

Table 1
Quality attributes of noodle (YAN) produced from wild-type (WT) and high-amylose wheat (HAW) flours. * indicates a significant difference between WT and HAW ($p < 0.05$).

		WT	HAW
Appearance	Yellowness (raw)	15.9 ± 1.2	16.1 ± 0.2
	Yellowness (cooked)	7.9 ± 0.7	13.4 ± 0.3 *
Texture of cooked YAN	Hardness (N)	5.0 ± 1.2	12.9 ± 1.3 *
	Springiness (%)	92.8 ± 2.1*	84.4 ± 2.1
	Cohesiveness (%)	75.3 ± 5.5*	52.6 ± 2.6
Resistant starch of cooked YAN (%)		1.2 ± 0.2	9.6 ± 1.3 *

3.2. Noodle digestibility

Evidence from resistant starch content (Table 1) and *in vitro* digestion (Fig. 1) showed cooked HAW noodle to be more resistant to digestion with an eight-fold higher resistant starch content (9.6%) than the wild type (1.2%). Newberry et al. (2018) reported a similar level of resistant starch content in the noodle made of HAW flour. It is noted that the values of resistant starch content could depend on noodle types and processing parameters (*e.g.*, squeezing could induce a more compact structure which was proposed to retard starch digestion in pasta (Zou et al., 2016)). The LoS plot (ln dC/dt) of the digestion curve is not linear, suggesting that there are at least two distinct digestion steps. A similar type of LoS plot was also reported for cooked pasta (Zheng, Stanley, Gidley, & Dhital, 2016; Zou et al., 2015).

3.3. Starch and gluten structure observed by CSLM

The morphology of cooked noodle strands (inner and outer section) was observed under CSLM with protein stained by fluorescamine and starch stained by FITC (Fig. 2). The inner section of noodles showed a lower extent of starch swelling and a relatively compact or dense microstructure, compared to the outer section. Starches in the outer section of WT appears to be completely gelatinized and forms a relatively homogenous network, while those of HAW largely retained granular structure (partially swollen and gelatinized) after the cooking process (100 °C, 10 min). Stained protein in HAW noodle showed regions with relatively stronger fluorescent intensity (indicated by arrows in Fig. 2), suggesting that relatively more dense local protein networks could be formed in HAW noodle than wild type. The observations explain the difference in noodle hardness (Table 1). In the inner section, there was granular structure retained even for WT starch. It is noted that CSLM only provides a qualitative comparison of starch gelatinization extent within the food matrix.

3.4. FTIR analysis

FTIR-ATR was used to characterise the protein components in flour and noodle samples in both raw and cooked forms (Fig. 3). All the samples were equilibrated to the same moisture content and subsequently analyzed at room temperature (~20 °C). It is noted that the samples derived from RS100 wheat flour showed a higher intensity of amide I band (1580 – 1720 cm^{-1}) (Li, Dobraszczyk, Dias, & Gil, 2006) than that of RS01. The observation is confirmed from measurement of higher protein contents in HAW flours than the wild type. Thus, the method using FTIR here could be useful for differentiating the relative protein content in wheat flours or (regions within) wheat-based food, particularly as cooked flour samples showed similar patterns as uncooked samples. This is consistent with the finding that the heat-induced changes of gluten in FTIR were mainly reversed after cooling down (Georget & Belton, 2006).

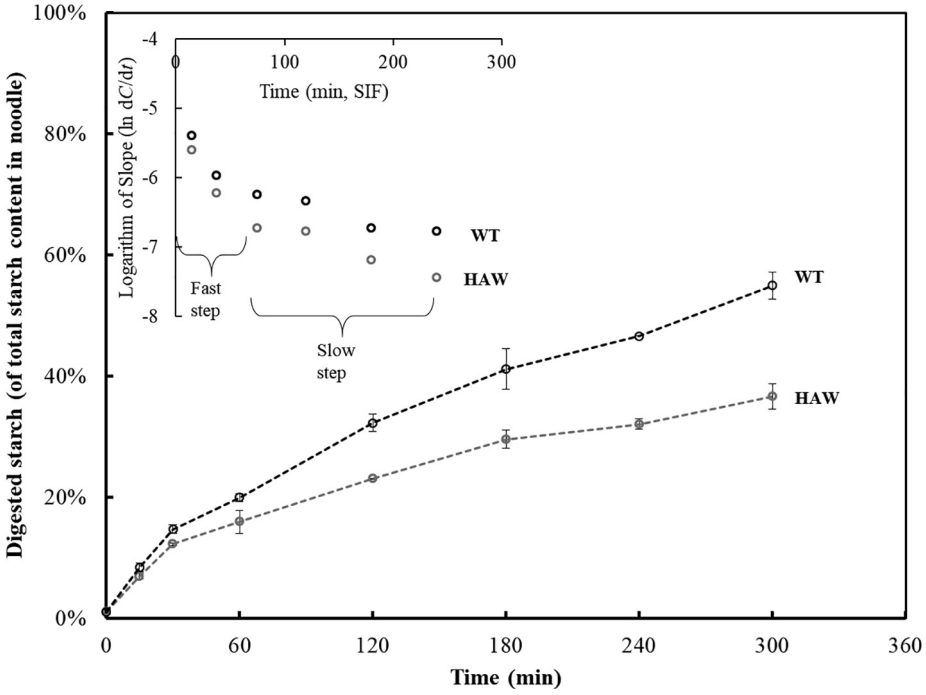

Fig. 1. Digestion of cooked noodles (YAN) produced from wild-type and high-amylose wheat flours and the plot of Logarithm of Slope as a function of time showing there are fast and slow steps of noodle digestion.

3.5. Starch digestion in protein-starch matrix

Through microstructural analysis of noodles, two forms of protein-starch matrix are identified, associated with different extents of starch swelling: the external regions of wild-type noodles have a lower level of structural integrity (*i.e.*, completely swollen starch forms a homogenous starch-gluten network), compared to HAW (*i.e.*, partly swollen starch granules are entrapped in more localised dense protein networks). Raw and cooked flours were used as representative materials to further investigate the effect of flour protein on starch digestibility.

Compared to wild type, more protein in HAW was hydrolysed by trypsin and chymotrypsin in the simulated intestinal fluid as shown in Fig. 4. The final digestion extent of flour protein ranks the same for native and cooked flours (RS100 > RS137 > RS01) and corresponds to the order of protein contents in the wheat flours. This result was used to select the time points (*i.e.*, 30 min and 4 h) of protein removal for testing its effect on starch digestibility.

Starch granules are found in association with the gluten network in wetted wheat flours (Figure S1). The protein components, mainly gliadins and glutenins, were stained by fluorescamine in red. An apparent protein network filled with starch granules is visible under CSLM. The protein matrix significantly reduced starch digestibility for all raw wheat flours (Fig. 5A–C). A 'lag' phase can be identified in the early stage of flour digestion, which was not found in the digestion of isolated starches (Li, Dhital, Gidley, et al., 2019). WT has a shorter time of the 'lag' phase (~60 min) than RS100 (~240 min). This is likely due to more protein encapsulation and interaction in RS100. Bhattarai et al. (2016) also reported that gluten addition to wheat starch retards starch digestion. After the pre-digestion of protein by trypsin and chymotrypsin in SIF, the barrier effect of proteins on starch hydrolysis was reduced. After cooking (Fig. 5D–F), the 'lag' phase was not found in the early stage of digestion.

Kinetic analysis (Table S2) showed that protein removal increased the rate coefficient (k) in both raw and cooked flour materials. The barrier effect of protein in raw flour materials was also reported previously (Bhattarai et al., 2016; Zhang & Hamaker, 1998). The extents of final digested starch percentage for cooked flours were similar between 4-h and control treatment (treated in enzyme-free buffer for 4 h). Zhang and Hamaker (1998) also found that protein digestion of cooked sorghum flour samples did not affect the final starch digestion extent. The results suggest that hydrothermal processing conditions applied to foods could largely eliminate the barrier effect of gluten proteins against starch digestion.

It is also noted that cooked wild-type flour after 30 min (time in SIF) protein removal has a higher final digested extent than 4-h and control samples. This is likely due to the extent of starch retrogradation for 30-min samples being less than for samples treated for a longer time of protein removal, with retrogradation enhancing starch resistance to digestion.

4. Discussion

There are various structural levels of starch-protein interactions in native grains, resulting from proteins located on (coating) granular surfaces and internal pores, and the discrete protein bodies deposited around starch in grain tissue (Dhital et al., 2019). The interaction of starch with protein in food ingredients (*e.g.*, wheat flour) and their response to processing have important implications for food quality and nutritional functionality.

4.1. Protein-starch matrix affects HAW noodle properties

The changes in noodle texture are in agreement with previous correlation analyses between amylose content and noodle eating qualities, including hardness, cohesiveness, and springiness (Heo, Baik, Kang, Choo, & Park, 2012; Kaur et al., 2016). Starch functionality in the food environment can be predicted using starch characterization techniques; Li, Dhital, Slade, et al. (2019) showed that starch swelling and interactions between swollen granules of HAW were greatly reduced at amylose levels higher than 50%, consistent with the observation of cooked HAW noodle under CSLM (Fig. 2). The difference in the structure of inner and outer regions of cooked noodles can be mainly attributed to the various extent of starch swelling and gelatinization. The morphological difference between these two regions probably underlies

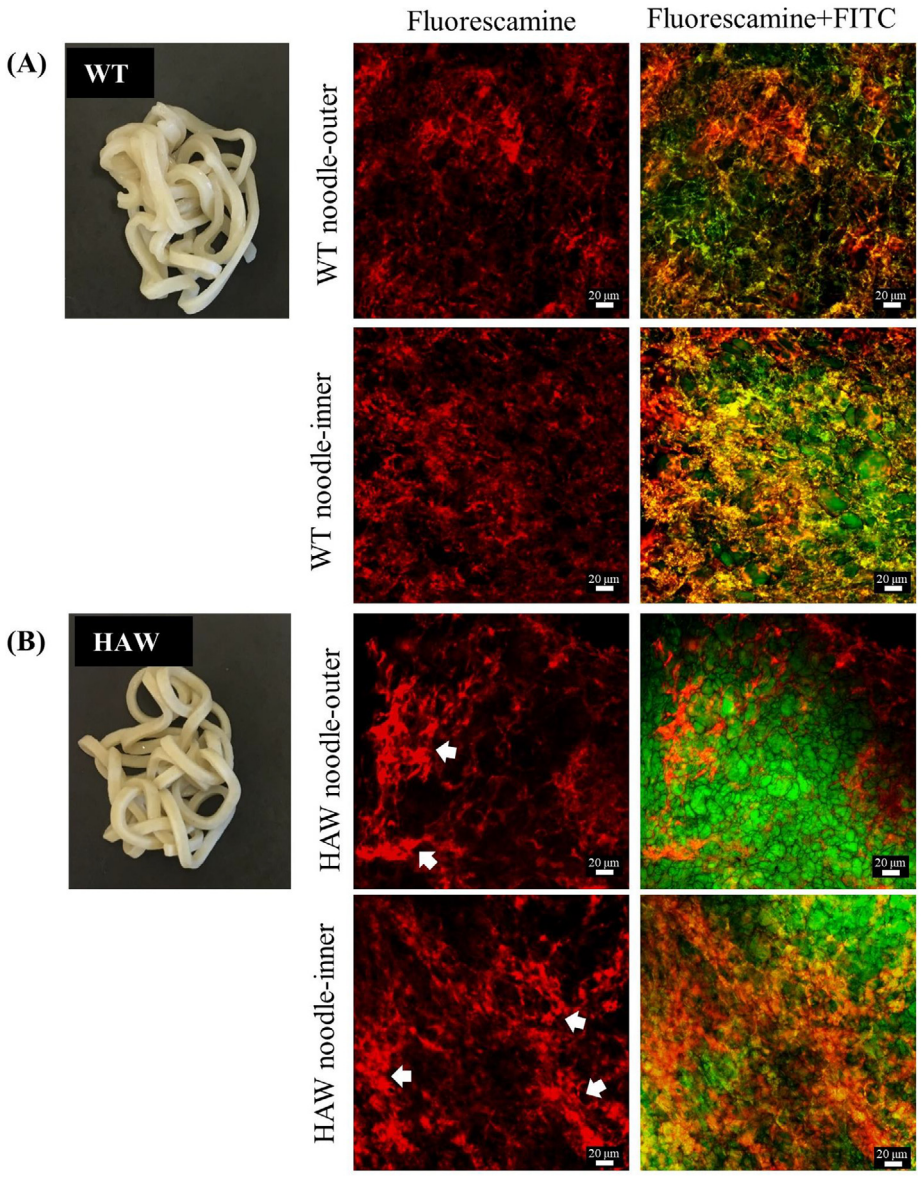

Fig. 2. Protein matrix in the inner and outer section of cooked noodles (YAN) produced from wheat wild type (A) and HAW (B). Protein matrix and starch were stained by Fluorescamine (red) and FITC (green), respectively. The confocal images have the same scale bar (20 μm). Arrows indicate regions with relatively higher fluorescent intensity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

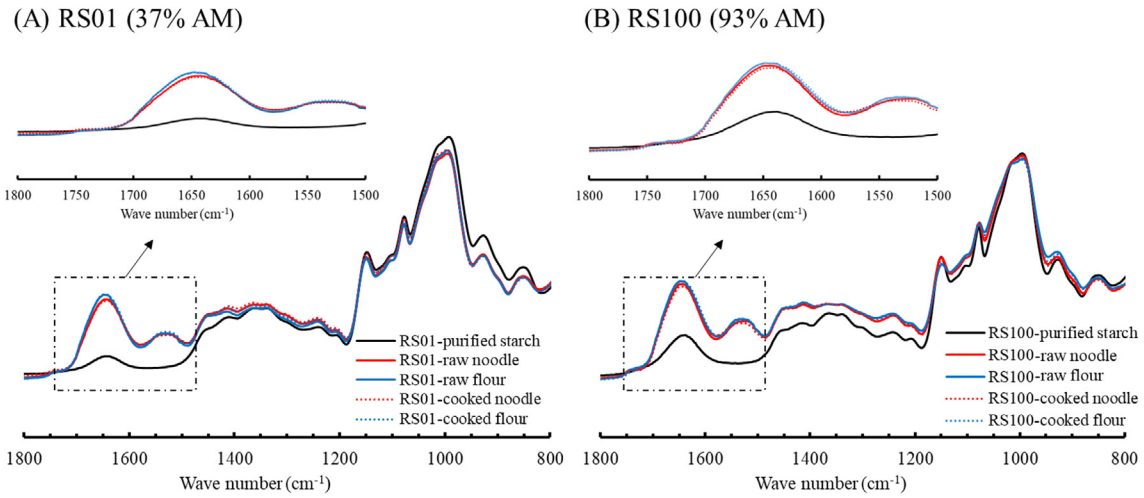

Fig. 3. FTIR–ATR spectra for wheat flours and noodle materials after SNV normalisation (A: wild type, B: HAW), with expansions of the amide-I band region.

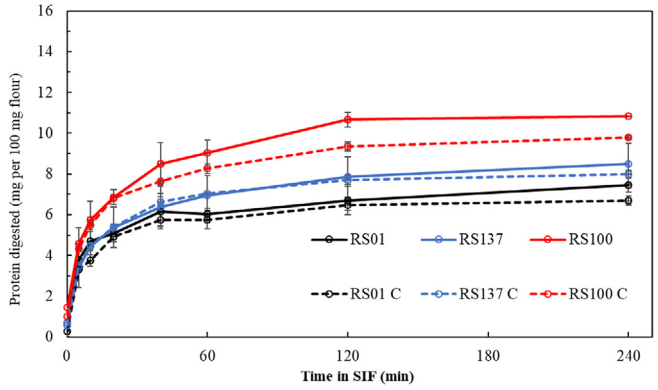

Fig. 4. Protein digestion of wheat flours (wild type RS01, HAW RS137 (47% AM) and HAW RS100 (93% AM)) in simulated digestion fluids (solid line: native form, dashed line: after cooking).

the LoS plot (Fig. 1) with slow and fast digestion steps for outer and inner sections, respectively.

Compared to the control, HAW noodle has relatively similar springiness, while hardness and cohesiveness showed more difference. Springiness describes the recovery process to un-deformed condition after the deforming force is removed. In addition to the starch gel network, the elasticity of the gluten matrix is also a factor that contributes to noodle springiness (Li et al., 2017; Veraverbeke & Delcour, 2002). In HAW noodle, the increased amount of gluten could compensate for the textural change due to the limited swelling ability of starch. However, it is not clear what the effect of other non-starch components (non-starch polysaccharides and lipids) in HAW on those parameters relevant to noodle texture properties is, and this needs further study.

4.2. Protein-starch matrix enhances the resistance against α-amylase digestion in HAW

In this study, wheat flours with various amylose content were used as a model food component to investigate starch-protein interactions in relation to amylose content. The gluten network can form spontaneously after adding water (Figure S1), and is an essential intermediate step to transform flours into foods. Such a food matrix of mainly starch and protein in wheat flours is relatively simple and homogenous, compared to food structures in noodle.

Wheat flours with and without cooking were used to represent the extreme ends of the scale of food-matrix forms to investigate the binary interaction. Starch digestion after protein removal consistently has a higher rate constant (k) compared to the control (Table S2). The result suggests that the macronutrient protein provides hindrances to digestive enzymes in both the raw and cooked starch-protein matrix. Comparing wheat flours with different amylose content, the resistant nature of HAW starch (RS100) retains lower digestibility than the wild type after the removal of proteins. The approach used here, starting from identification of complex food structures to assays on representative

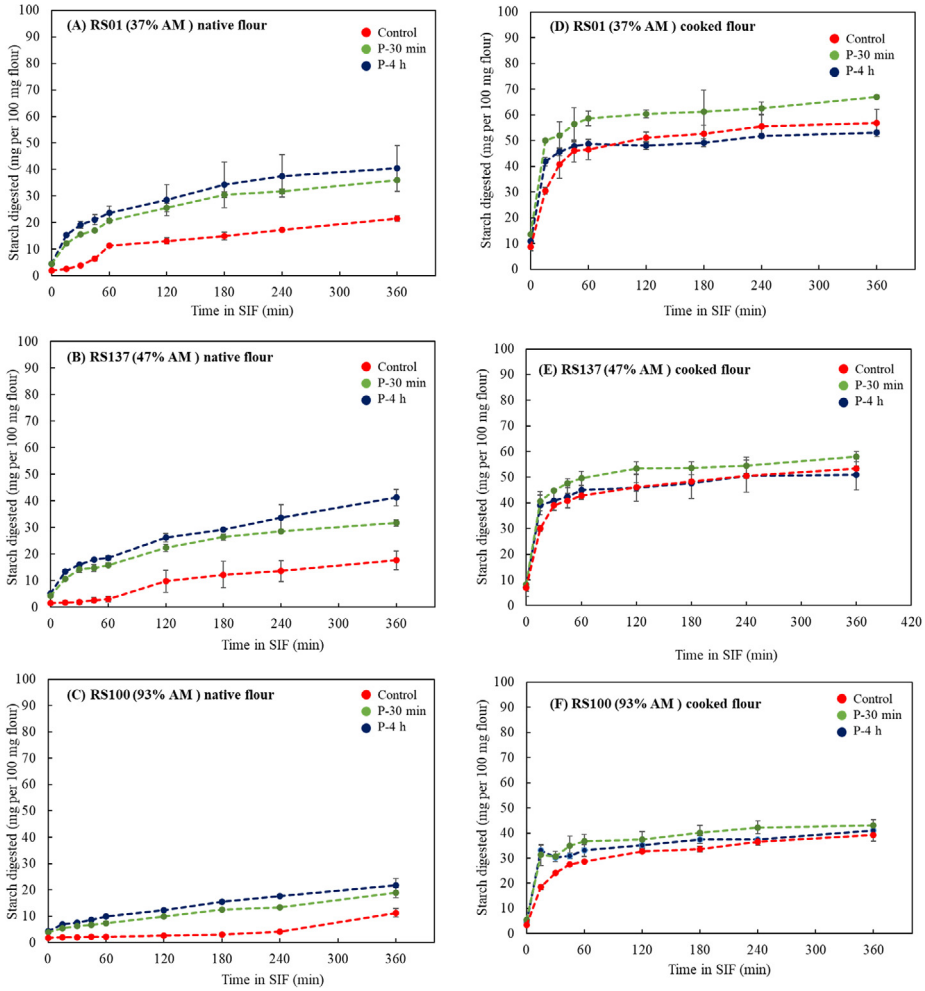

Fig. 5. Starch hydrolysis of native (A-C) and cooked (D-F) wheat flours in simulated small intestinal fluid (A & D: RS01, B & E: RS137, C & F: RS100) after protein predigestion for 30 min (P-30 min) and 4 h (P-4 h). Control: treated with digestion fluids (without enzymes added).

forms of wheat flours as a model, could allow a better understanding of the synergistic effects in a real food environment. This study indicates that interactions between starch and protein in whole food can modulate the *in vitro* hydrolysis of macronutrients. This could be the mechanistic basis resulting in a higher glycemic response of gluten-free bread, compared to regular bread (Berti et al., 2004; Jenkins et al., 1987).

In wheat flour, the matrix after hydration consists of starch granules and gluten, essentially formed by uncross-linked monomeric gliadins and polymeric (cross-linked) glutenins. Stable interactions with starch could be developed through hydrogen bonding and hydrophobic effects (Dhital et al., 2019). Surface proteins of starch granules could play an intermediary role by complexing with other proteins through charge-charge interactions, which is pH-dependent (Dhital et al., 2019; Marshall & Chrastil, 1992). The gluten network has been shown to effectively limit α-amylase hydrolysis here and in previous studies. The gluten network has pores at the μm length scale (Fardet, Hoebler, Djelveh, & Barry, 1998) and would not be expected to completely prevent the diffusion of α-amylase (~4 nm) (Payan et al., 1980) as a physical barrier. Thus, binding of α-amylase to gluten could also contribute to the inhibition of starch digestion, as proposed previously (Bhattarai et al., 2016; Zou et al., 2015). The increased protein content in native HAW flours (Table S1) could give more innate resistance to amylolysis, in addition to higher enzymatic resistance of starch substrate *per se* that is generally reported for high-amylose starches (Li, Gidley, et al., 2019a).

The cooking process was found to minimize the inhibition of enzymatic action on the starch substrate. Hydrothermal treatment with excess water denatures protein and causes starch gelatinization. During cooking, a continuous protein network can be formed with extensive cross-linking (Marshall & Chrastil, 1992). At the same time, glucan polymers can be released after the bursting of swollen granules or be entrapped within the gluten network. The unchanged final digestion extent after protein removal is likely due to the increased accessibility of the substrate after cooking. Starch swelling enlarges the surface area for enzyme binding as well as disrupting the protein network. Gelatinized starch materials may be less restricted within the disrupted protein network, as amylose and amylopectin are neutral molecules, with only limited interactions after gelatinization. However, for HAW starch with higher thermal stability as characterised previously (Li, Dhital, Slade, et al., 2019), it is reasonable to assume that the destruction of the granule structure after heating is relatively limited. This implies that the increased amylose level in starch-based foods not only enhances enzymatic resistance of the substrate, but also allows complex food matrices to be retained during cooking, leading to more resistance against digestive enzymes at the whole food level.

It is noted that there are limitations in the ability to link the results of wheat flour to the final food environment. The starch-protein matrix in wheat flour is relatively homogenous without variation in different fractions or regions as found in noodles. While using flours as a model allows the effect of cooking and amylose levels on the digestibility of the whole food to be assessed, the local density in food could not be captured. In food, the final density depends on processing such as kneading, sheeting and extruding, etc., which have an impact on starch functionality (BeMiller, 2011) and digestibility (Zhang, Dhital, & Gidley, 2015).

5. Conclusion

HAW has an eight-fold increase of resistant starch content in noodle, along with increased hardness and reduced springiness and cohesiveness. Two distinct forms of protein-starch matrix are observed in the external region and strand centre of cooked noodles. To further understand starch-protein interaction in wheat-based foods in relation to starch digestibility, wheat flours were used as a model in a reductionist approach: two forms of wheat flours (native and cooked) representing the range of food matrices identified in noodle strands. The interaction between starch and protein (*i.e.*, gluten network physically entraps starch granules) in wheat flours provides inhibition of hydrolysis regardless of the native or cooked form. The increased protein content and thermal stability of starch in HAW flour contributes to the food integrity in HAW noodle, and consequently the resistance against α-amylase digestion. The results support the concept that complex food matrices in whole food could be more nutritious and healthier, compared to those with isolated nutrients. Overall, the study demonstrates that the protein-starch matrix in HAW underlies the changes in noodle properties and enhances the resistance against α-amylase digestion.

CRediT authorship contribution statement

Hai-Teng Li: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Zaifen Li:** Investigation, Formal analysis. **Glen P. Fox:** Investigation, Writing - review & editing. **Michael J. Gidley:** Supervision, Writing - review & editing, Funding acquisition. **Sushil Dhital:** Supervision, Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127719.

References

Baik, B. K., & Lee, M. R. (2003). Effects of starch amylose content of wheat on textural properties of white salted noodles. *Cereal Chemistry, 80*(3), 304–309.
BeMiller, J. N. (2011). Pasting, paste, and gel properties of starch–hydrocolloid combinations. *Carbohydrate Polymers, 86*(2), 386–423.
Berti, C., Riso, P., Monti, L. D., & Porrini, M. (2004). *In vitro* starch digestibility and in vivoglucose response of gluten–free foodsand their gluten counterparts. *European Journal of Nutrition, 43*(4), 198–204.
Bhattarai, R. R., Dhital, S., & Gidley, M. J. (2016). Interactions among macronutrients in wheat flour determine their enzymic susceptibility. *Food Hydrocolloids, 61*, 415–425.
Bhattarai, R. R., Dhital, S., Mense, A., Gidley, M. J., & Shi, Y.-C. (2018). Intact cellular structure in cereal endosperm limits starch digestion in vitro. *Food Hydrocolloids, 81*, 139–148.
Brennan, C. S., Harris, N., Smith, D., & Shewry, P. R. (1996). Structural differences in the mature endosperms of good and poor malting barley cultivars. *Journal of Cereal Science, 24*(2), 171–177.
Debet, M. R., & Gidley, M. J. (2006). Three classes of starch granule swelling: Influence of surface proteins and lipids. *Carbohydrate Polymers, 64*(3), 452–465.
Dhital, S., Bhattarai, R. R., Gorham, J., & Gidley, M. J. (2016). Intactness of cell wall structure controls the *in vitro* digestion of starch in legumes. *Food and Function, 7*(3), 1367–1379.
Dhital, S., Brennan, C., & Gidley, M. J. (2019). Location and interactions of starches in planta: Effects on food and nutritional functionality. *Trends in Food Science and Technology, 93*, 158–166.
Ding, L., Huang, Q., Li, H., Wang, Z., Fu, X., & Zhang, B. (2019). Controlled gelatinization of potato parenchyma cells under excess water condition: Structural and *in vitro* digestion properties of starch. *Food and Function, 10*, 5312–5322.
Fardet, A., Hoebler, C., Djelveh, G., & Barry, J.-L. (1998). Restricted bovine serum albumin diffusion through the protein network of pasta. *Journal of Agricultural and Food Chemistry, 46*(11), 4635–4641.
Fardet, A., & Rock, E. (2014). Toward a new philosophy of preventive nutrition: From a

reductionist to a holistic paradigm to improve nutritional recommendations. *Advances in Nutrition (Bethesda, Md.), 5*(4), 430–446.
Georget, D. M. R., & Belton, P. S. (2006). Effects of temperature and water content on the secondary structure of wheat gluten studied by FTIR spectroscopy. *Biomacromolecules, 7*(2), 469–475.
Guo, G., Jackson, D., Graybosch, R., & Parkhurst, A. (2003). Asian salted noodle quality: Impact of amylose content adjustments using waxy wheat flour. *Cereal Chemistry, 80*(4), 437–445.
Hamaker, B. R., & Griffin, V. K. (1993). Effect of disulfide bond-containing protein on rice starch gelatinization and pasting. *Cereal, Chemistry(4)*, 377–380.
He, H., & Hoseney, R. C. (1992). Effect of the quantity of wheat flour protein on bread loaf volume. *Cereal Chemistry, 69*(1), 17–19.
Heo, H., Baik, B.-K., Kang, C.-S., Choo, B.-K., & Park, C. S. (2012). Influence of amylose content on cooking time and textural properties of white salted noodles. *Food Science and Biotechnology, 21*(2), 345–353.
Jenkins, D. J., Thorne, M. J., Wolever, T. M., Jenkins, A. L., Rao, A. V., & Thompson, L. U. (1987). The effect of starch-protein interaction in wheat on the glycemic response and rate of in vitro digestion. *The American Journal of Clinical Nutrition, 45*(5), 946–951.
Kaur, A., Shevkani, K., Katyal, M., Singh, N., Ahlawat, A. K., & Singh, A. M. (2016). Physicochemical and rheological properties of starch and flour from different durum wheat varieties and their relationships with noodle quality. *Journal of Food Science and Technology, 53*(4), 2127–2138.
Konik, C. M., Miskelly, D. M., & Gras, P. W. (1993). Starch swelling power, grain hardness and protein: Relationship to sensory properties of Japanese noodles. *Starch - Stärke, 45*(4), 139–144.
Li, H., Dhital, S., Gidley, M. J., & Gilbert, R. G. (2019). A more general approach to fitting digestion kinetics of starch in food. *Carbohydrate Polymers, 225*, Article 115244.
Li, H., Dhital, S., Slade, A. J., Yu, W., Gilbert, R. G., & Gidley, M. J. (2019). Altering starch branching enzymes in wheat generates high-amylose starch with novel molecular structure and functional properties. *Food Hydrocolloids, 92*, 51–59.
Li, M., Dhital, S., & Wei, Y. (2017). Multilevel structure of wheat starch and its relationship to noodle eating qualities. *Comprehensive Reviews in Food Science and Food Safety, 16*(5), 1042–1055.
Li, W., Dobraszczyk, B. J., Dias, A., & Gil, A. M. (2006). Polymer conformation structure of wheat proteins and gluten subfractions revealed by ATR-FTIR. *Cereal Chemistry, 83*(4), 407–410.
Li, H., Gidley, M. J., & Dhital, S. (2019a). High-amylose starches to bridge the "fiber gap": Development, structure, and nutritional functionality. *Comprehensive Reviews in Food Science and Food Safety, 18*(2), 362–379.
Li, H., Gidley, M. J., & Dhital, S. (2019b). Wall porosity in isolated cells from food plants: Implications for nutritional functionality. *Food Chemistry, 279*, 416–425.
Marshall, W. E., & Chrastil, J. (1992). Interaction of Food Proteins with Starch. In B. J. F. Hudson (Ed.). *Biochemistry of Food Proteins* (pp. 75–97). Boston, MA: Springer, US.
Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., ... Brodkorb, A. (2014). A standardised static *in vitro* digestion method suitable for food an international consensus. *Food and Function, 5*(6), 1113–1124.
Morita, N., Maeda, T., Miyazaki, M., Yamamori, M., Miura, H., & Ohtsuka, I. (2002). Dough and baking properties of high-amylose and waxy wheat flours. *Cereal Chemistry, 79*(4), 491–495.
Newberry, M., Berbezy, P., Belobrajdic, D., Chapron, S., Tabouillot, P., Regina, A., & Bird, A. (2018). High-amylose wheat foods: A new opportunity to meet dietary fiber targets for health. *Cereal Foods World, 63*(5), 188–193.
Payan, F., Haser, R., Pierrot, M., Frey, M., Astier, J. P., Abadie, B., ... Buisson, G. (1980). The three-dimensional structure of α-amylase from porcine pancreas at 5 Å resolution – the active-site location. *Acta Crystallographica Section B, 36*(2), 416–421.
Van Hung, P., Yamamori, M., & Morita, N. (2005). Formation of enzyme-resistant starch in bread as affected by high-amylose wheat flour substitutions. *Cereal Chemistry, 82*(6), 690–694.
Veraverbeke, W. S., & Delcour, J. A. (2002). Wheat Protein Composition and Properties of Wheat Glutenin in Relation to Breadmaking Functionality. *Critical Reviews in Food Science and Nutrition, 42*(3), 179–208.
Warren, F. J., Gidley, M. J., & Flanagan, B. M. (2016). Infrared spectroscopy as a tool to characterise starch ordered structure—a joint FTIR–ATR, NMR, XRD and DSC study. *Carbohydrate Polymers, 139*, 35–42.
Zhang, B., Dhital, S., & Gidley, M. J. (2015). Densely packed matrices as rate determining features in starch hydrolysis. *Trends in Food Science and Technology, 43*(1), 18–31.
Zhang, G., & Hamaker, B. R. (1998). Low α-amylase starch digestibility of cooked sorghum flours and the effect of protein. *Cereal Chemistry, 75*(5), 710–713.
Zhang, G., & Hamaker, B. R. (2003). A three component interaction among starch, protein, and free fatty acids revealed by pasting profiles. *Journal of Agricultural and Food Chemistry, 51*(9), 2797–2800.
Zheng, Z., Stanley, R., Gidley, M. J., & Dhital, S. (2016). Structural properties and digestion of green banana flour as a functional ingredient in pasta. *Food and Function, 7*(2), 771–780.
Zou, W., Sissons, M., Gidley, M. J., Gilbert, R. G., & Warren, F. J. (2015). Combined techniques for characterising pasta structure reveals how the gluten network slows enzymic digestion rate. *Food Chemistry, 188*(Supplement C), 559–568.
Zou, W., Sissons, M., Warren, F. J., Gidley, M. J., & Gilbert, R. G. (2016). Compact structure and proteins of pasta retard in vitro digestive evolution of branched starch molecular structure. *Carbohydrate Polymers, 152*, 441–449.