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The structural integrity of endosperm/cotyledon cells and cell modification affect starch digestion properties

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Nutritional and epidemiological studies suggest that the excessive intake of highly processed starchy foods contributes to the risk of type II diabetes and obesity in consumers. This is partly caused by the disruption of the cellular structure of cereal endosperms or legume cotyledons in foods during processing, which releases large amounts of highly digestible starch though the cell wall structure. Thus, to improve the production of starch-based foods with slowly digestible starch, it is necessary to clarify the influence of the structural integrity of cereal endosperm and legume cotyledon cells and the modification of their structure during processing on the starch digestion properties. However, the effect of mechanical, chemical, biological, or enzymatic modification of the cell wall during the processing of cereals and legumes on the digestive properties of starch has not been summarized well. Accordingly, in the present review, we fill this gap by summarizing the biophysical properties of common cereal and legume endosperm/cotyledon cells. Furthermore, we elaborate on the mechanisms involved in imparting cell wall integrity and controlling the starch digestion properties. Subsequently, the starch release pattern after cell wall modification is also discussed. In addition, a new classification system is proposed, which is beneficial for conducting cell research. This review provides new insights into the cell wall integrity of starch sources and the effect of the modification of cereal and legumes on starch digestion, which will benefit the scientific community and industry.

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1. Introduction

Starch is stored in cereal endosperm and legume cotyledon cells, where it is the main carbohydrate[1] with a complex hierarchical structure containing glucose units, double helices, crystalline and amorphous thin layers, growth rings, and intact starch granules.[2,3] The digestive properties of starch are one of its most important functional properties, given that starch is converted to glucose after digestion and absorbed in the intestinal mucosa, thus resulting in an increase in the blood glucose level.[4] Cereal and legume products exhibit a wide glycemic index (GI). It has been reported that the excessive consumption of foods with a high GI can induce the development of obesity or type II diabetes.[5,6] Thus, to reduce the development of these diseases, it is necessary to control glycemia. The main strategy for controlling the glycemic response aims to reduce the amount of carbohydrates available for digestion and the rate of glucose absorption, while increasing the rate of glucose removal from the blood.[7]

Starch from different species of cereals or legumes presents varying starch digestibility, which is related to the differences in the physiochemical properties of starch. The morphology (size and shape) of starch granules, their specific surface area, form of polymerization, and the ratio of amylose to amylopectin are all known to influence the starch digestibility.[8] For certain species of cereals or legumes, different extents of structural integrity also contribute to the various starch digestibility. For example, whole grain foods and coarsely processed cereal or legume products have lower glycemic response values compared to refined products, which is primarily due to the integrity of the food structure. Unrefined cereal and legume products retain intact cellular structures, such as the cell wall, which acts as a barrier to the complete gelatinization or fast digestion of starch.[9] In the 1980s, pioneering research showed that particle size also affects the digestibility of starch and refined flours increase glycemia and insulinemia more than the coarse ones.[10] The association between the structural integrity of cereal and digestibility of starch was described in the 1990s, which also highlighted that cellular-level differences in cereal kernels result in varying structural integrity.[11]

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However, the importance of the cellular structure was only systematically investigated several decades after its potential role in digestibility was proposed. In 2015, Edwards *et al.*[12] showed that the structural integrity of wheat endosperm cells was the primary factor influencing the bioaccessibility of their starch, indicating that the modulation of the structural integrity of endosperm cells plays a crucial role in postprandial glucose metabolism. This study heralds a new era of research on the regulation of the digestibility of starch from cereal endosperm cells and elevates the understanding of the relationship between tissue damage during the milling and processing of grains and its effect on starch digestibility. These are important issues that must be brought to the forefront of international grain processing research.

In this review, we summarize the mechanisms by which endosperm cell structure affects the digestibility of starch in both intact and broken systems. We highlight the modification of endosperm cells caused by a series of operations during the processing of cereals and discuss how these modifications may affect the digestibility of starch. In addition, we also provide an outlook on the priorities and urgent issues in this field of research that should be addressed in relevant future research.

2. Cellular architecture and chemical composition of cereal endosperms/legume cotyledons

2.1. Structure of cells in cereal endosperms/legume cotyledons

Starch is abundant in the endosperm cells of monocotyledonous cereals (wheat, rice, oats, barley, sorghum, *etc.*) and the cells of dicotyledonous legumes (chickpeas, kidney beans, mung beans, *etc.*).[13,14] Thus, it is important to clarify the cellular structure in endosperms and cotyledons to investigate its relationship with starch digestion properties. The size, porosity and permeability of the cell wall, and intracellular nutrient content of endosperm/cotyledon cells need to be investigated. These data are expected to be important in studying the relationship between cell structure and starch digestion readiness.

Fig. 1 shows the microstructure of cross-sections of wheat, rice, and maize endosperms stained with Fluorescent Brightener 28 under a fluorescence microscope. It can be noted that the cells of the cereal endosperm show an irregular polygonal morphology and are closely aligned with each

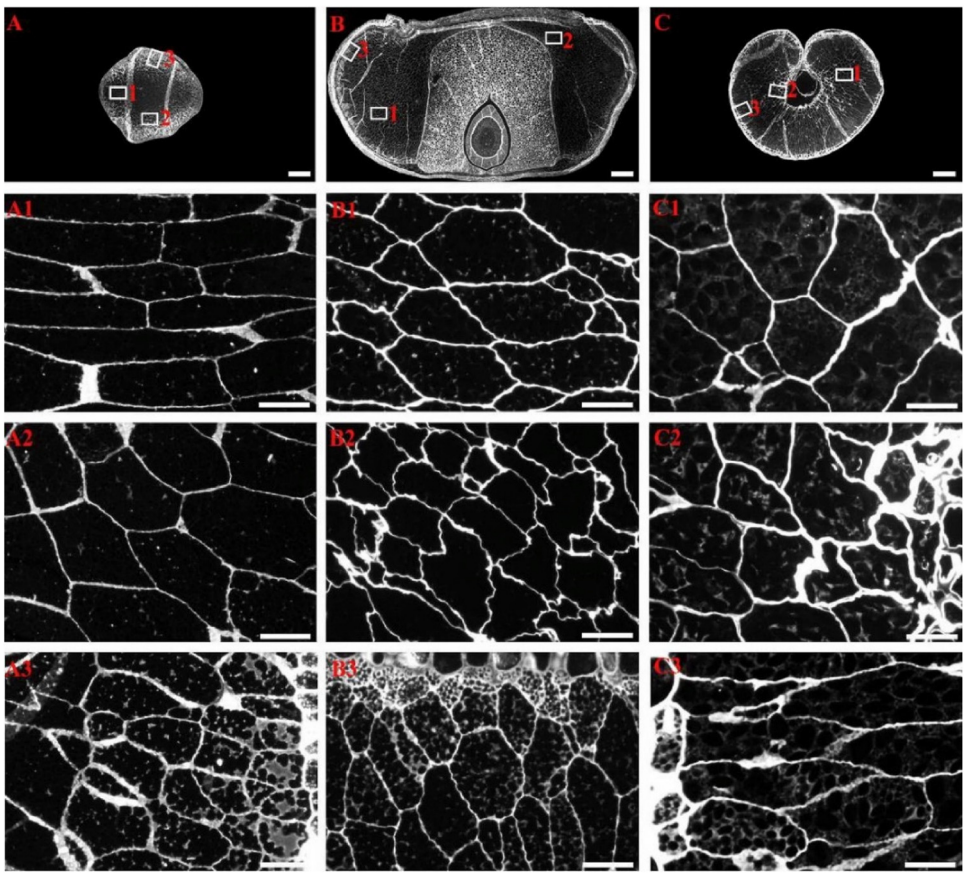

Fig. 1 Fluorescence micrographs of cross-sections of the intact seed of mature rice (A), maize (B), and wheat (C) after staining with Fluorescent Whitening Agent 28. (A1–3, B1–3, and C1–3) Cell morphology of three different regions in the endosperm of rice, maize, and wheat, where region 1 for each species is shown in A1, B1, and C1; region 2 in A2, B2, and C2; and region 3 in A3, B3, and C3, respectively. The scale bar is 500 μm for (A–C) and 50 μm for (A1–C3). These graphs were collected from Zhao *et al.*[15]

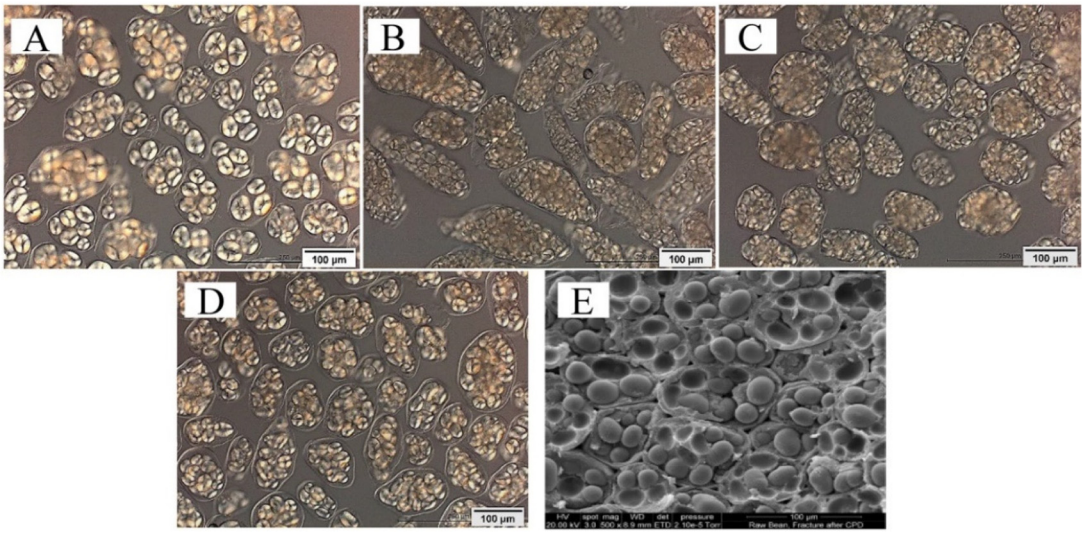

Fig. 2 DIC (differential interference contrast) light micrograph of isolated cotyledon cells (A–D) and scanning electron micrographs showing sections of navy bean cotyledon cells (E). Isolated cotyledon cells of adzuki bean (A), chickpea (B), lentil (C), and Lima bean (D). The scale bar is 100 μm for (A–E). These graphs are modified from Duc Toan *et al.*[16] and Berg *et al.*[17]

another. In this study, three regions were selected for observation based on the distance of the cells from the center and edge of the endosperm (outer edge of the endosperm (region 1) – A1, B1, and C1; center of the endosperm (region 2) – A2, B2, and C2; and inner edge of the endosperm (region 3) – A3, B3, and C3).[15] There are differences in the size and shape of the cells in different regions of the endosperm, with the rice and maize endosperm cells being the smallest in region 3 and wheat endosperm cells being the smallest in region 2. The cells in the center of the endosperm (region 1) have a more regular morphological profile, whereas the cells at the edge of the endosperm (regions 2 and 3) present irregular cell shapes.[15] Fig. 2A–D depict images of isolated cotyledon cells of an adzuki bean, chickpea, lentil, and Lima bean, respectively, captured using light microscopy in the differential interference contrast (DIC) mode. It can be seen that the size and shape of the cotyledon cells differ significantly among the different legumes, with the chickpea cells tending to be elliptical, while the cells of the other three legumes are generally spherical in shape. In addition, Fig. 2A–D also show the morphology of the intracellular starch granules, that the size of the starch granules differs significantly among the beans, and the size of the starch granules in the cotyledon cells of the adzuki bean is significantly larger than that in the cells of the other three beans.[16] Fig. 2E shows a cross-sectional electron micrograph of the cotyledons of navy beans, which shows that the cotyledon cells of legumes are also closely packed, similar to the cells in the cereal endosperm structure.[17]

Table 1 shows the endosperm/cotyledon cell sizes of common cereals and legumes, with the particle size (by long axis length) of the common monocotyledonous cereal endosperm cells ranging from roughly 80 to 120 μm and the cotyledon cell size of common dicotyledonous legumes from 98 to 117 μm.

2.2. Chemical composition of cereal endosperm/legume cotyledon cells

2.2.1. Starch. Starch is the most abundant nutrient in cereal endosperm and legume cotyledons. Starch has a complex hierarchical structure, where its molecules consist of α-D-glucopyranosyl units linked by glycosidic bonds to long-chain glucan macromolecules. The glucan chains are intertwined in native starch to form a double helical structure and the helices form a lamellar structure consisting of ordered crystalline and amorphous regions. This lamellar structure occupies most of the space inside the starch granule. These starch granules are usually embedded in the protein matrix and encapsulated by the cell wall in the endosperm cells of cereals or the cotyledon cells of legumes.[18–20] Table 1 shows the percentage of starch, protein, and lipid content in the endosperm/cotyledon cells of common cereals and legumes. It can be seen that starch dominates the cells, and the percentage of starch in the endosperm of rice even reaches 90%.

Most common cereal endosperm starches contain about 72–78% amylopectin and 18–33% amylose, and their structural features lead to differences in sensitivity to amylase.[21] Amylose is a linear molecule consisting of α-D-glucopyranosyl units linked by (1 → 4) glycosidic bonds, which has some branched chains linked by (1 → 6) bonds. Amylopectin is defined as starch containing highly branched components with chains of α-D-glucopyranosyl residues, which are mainly linked by (1 → 4) bonds but with 5–6% of the bonds are also (1 → 6) connections at the branching points.[18]

Based on the X-ray diffraction pattern of starch, it is mainly classified into three polymerization forms, *i.e.*, A, B, and C type. The different crystal structures of starch are due to the differences in the structure of its double helix. Furthermore, differences in its polymerization affect its hydrolysis. For

Table 1 Cell wall-related data for common cereals and legumes

Category	Species	Cell size/μm	Cell wall porosity/ nm	Cell wall thickness/ μm	Cellular composition (% of total endosperm/cotyledons)			Ref.
					Starch	Protein	Lipids	
Monocotyledon (cereal)	Wheat (*Triticum aestivum* L.)	80 (L) × 60 (W)	1.5–3	ND	75.00	14.00	1.00–3.00	37 and 64
	Rice (*Oryza sativa*)	90 (L) × 50 (W)	ND	ND	90.00	5.00–7.00	0.50–1.00	64 and 106
	Oat (*Avena sativa* L.)	ND	ND	ND	55.00	15.00–20.00	7.00	107
	Maize (*Zea mays* L.)	120 (L) × 70 (W)	1–2	ND	~70.00	~7.00	~0.70	38, 64 and 108
	Barley (*Hordeum vulgare* L.)	80 (L) × 60 (W)	ND	0.30	~68.00	~10.00	~1.00	64, 109 and 110
	Sorghum (*Sorghum bicolor* L. *Moench*)	90 (L) × 50 (W)	3.44 ± 0.49 (D)	ND	ND	ND	ND	39 and 64
	Millet (*Setaria italica var.*)	ND	3.44 ± 0.49 (D)	ND	ND	ND	ND	39
Dicotyledon (legumes)	Chickpea (*Cicer arietinum* L.)	117.7	3.5–5.5 (D)	~1–2	~40.00–50.00	~20.00–30.00	2.46	16, 41, 58 and 111
	Kidney beans (*Phaseolus vulgaris*)	100.0	ND	ND	40.00	27.50	1.65	16 and 112
	Lima bean (*Phaseolus lunatus*)	98.9	ND	~2.1–4.6	~82.0	17.3	0.10	16
	Adzuki bean (*Vigna angularis*)	102.4	ND	~4.1–6.3	~79.4	19.8	0.31	16

L: length; *W*: width; *D*: diameters; ND: No data. (a) Cell size without *L*/*W* denotes the average diameter of cells. (b) Cell wall porosity values that are not marked with a *D* denote the radius length.

example, A-type starch has shorter double helices with interior crystallites, and thus it is more readily hydrolyzed by amylase.[19]

2.2.2. Protein. Protein in cereals and legume seeds is the main source of protein for direct human consumption *via* food.[22] As shown in Table 1, the percentage of protein in the endosperm of common cereals is ~5–20%, while that in the cotyledons of common legumes is ~20%, making protein the second largest macronutrient group after starch in cereals and legumes. Storage proteins in grains form discontinuous protein bodies during grain filling and coalesce into a continuous protein matrix around starch granules in later developmental stages.[23] It has been reported that storage proteins in the endosperm also affect the digestive properties of starch. These proteins may reduce enzyme activity through starch-protein interactions or by providing a physical barrier to the diffusion of α-amylase into starch granules.[24]

2.2.3. Lipids. The lipid content in cereal endosperms and legume cotyledons is low, as shown in Table 1. The endosperms of common cereals contain 1.00–3.00% of the lipid content. Only oats have a high lipid content of about 7%, while legume cotyledons (non-oil crops) generally contain ~1% of lipid content. Lipids in cereals can surround starch granules, where they are loosely associated on the surface, and thus called starch surface lipids. They may also interact with amylose during starch pasting, which are called internal starch lipids, or appear in native starch granules and play an important role in the physicochemical properties of starch.[25,26] Typically, X-ray diffraction patterns indicate the presence of A-type or B-type starch in cereal and C-type starch in legume, whereas V-type patterns are characteristic of starch-lipid complexes.[24,27,28] The formation and content of V-type complexes between lipids and amylose affect the digestive properties of starch and contribute to a lower postprandial glycemic response.[29,30]

2.3. Structure and composition of cell wall in cereal endosperms/legume cotyledons

The cell wall is an important structural feature in plants, where it provides the necessary mechanical support for plant growth and development and protects plants from adverse environmental conditions such as biotic and abiotic stresses. In addition, in the case of edible plants, the cell wall is a common source of fiber in the human diet. Furthermore, the cell wall encapsulates a large number of nutrients involved in intracellular digestive processes.[31,32] Therefore, it is important to clarify the structure and chemical composition of the cell wall to study its effects on food properties. The structure and chemical composition of the cell wall in cereal endosperm and legume cotyledon cells have been described in detail in recent reviews.[32] In brief, the cell wall is mainly composed of cellulose and hemicellulose, and has a complex multi-layered structure.[33] Detailed data on the chemical composition and structure of the endosperm and cotyledon cells in cereals and legumes can be found in the report by Xiong *et al.*[32]

Comprehensive reports are available on the structure and chemical composition of the cell wall; specifically, a detailed model of the cell wall of wheat endosperm was reported by Gartaula *et al.*[34] However, a summary of the biophysical properties of the cellular structure in cereal endosperms and legume cotyledons is lacking. The thickness of the cell wall and the size of its pores affects the rate of intracellular and extracellular material transfer.[35] These differences in biophysical properties may be related to the rate at which enzymes cross the cell wall during digestion. The metabolic reactions of nutrients in endosperm/cotyledon cells are related to the porosity of the cell wall, which affects the permeability of digestive enzymes.[32] According to Table 1, the difference in the porosity of the cell wall among different types of cereals is not significant. It has been reported that the typical diameter of the pores in cell wall of crops is 2–8 nm.[36] The pores in the non-lignified cell wall in wheat was measured to be 1.5–3 nm in radius using approaches based on gas adsorption,[37] but no accurate data are available on the pore size in the cell wall of endosperms. The radius of the pores in the cell wall of maize is in the range of 0.5–5 nm according to the nitrogen adsorption method, and the majority of voids are 1–2 nm in radius.[38] Adani *et al.*[39] measured the majority of pores in the cell wall of millet and sorghum using the gas adsorption method, which were found to be in the range of 3.50–5.20 nm, where pores a diameter of 3.44 ± 0.49 nm were predominant. It is important to note that the cell wall porosity of the endosperm was not measured separately in the aforementioned study, and therefore the porosity data can only be used as a reference at this time. In addition, pore diameters in the range of 3.5–5.2 nm were measured in the cell wall of wheat using a solute exclusion technique, which differed from the diameters of 3–6 nm measured by the gas adsorption method.[36,40] Therefore, the porosity may differ across studies, which is worth considering when comparing the differences in porosity among different species of cereal.

The cells in legume cotyledons are non-woody cells. Generally, the diameters of the pores in the cell wall of non-woody legumes are in the range of 3.5 to 5.5 nm (Table 1).[41] Although no studies have been conducted to precisely measure the cell wall porosity of common legumes, studies on chickpeas have found that intact cells can impede the passage of α-amylase, which has a hydrodynamic diameter of approximately 8 nm.[42] Therefore, theoretically, the diameters of the pores in the cell wall of cotyledons should be in the range of 3.5–5.5 nm. In addition to the physical size of the pores in cell wall structures, α-amylase effectively binds to cellulose,[43] which is a common constituent of the cell wall of all cereals and legumes. This is an alternative/additional mechanism by which the cell wall can prevent the passage of amylase.

The thickness of the cell wall also affects the digestion of starch, which is in the range of 0.1–1 μm in legumes, while it is less than 1 μm in cereals, suggesting that legumes can withstand a greater degree of damage during processing and digestion and are more resistant to enzyme permeation than cereals. The cell wall thickness of chickpea cotyledons is ~1–2 μm, while that of barley endosperm has been reported to be 0.3 μm (Table 1). However, no accurate data is available for the cell wall thickness in other common legumes and cereals. We analyzed the microstructure images in some available reports using the ImageJ software.[16] The thickness of the cell wall of the cotyledon in Lima and adzuki beans was inferred (Table 1). However, this data can only be used as a reference, and the precise thickness of the cell wall must be ascertained by more systematic experiments.

3. The effect of the cell wall integrity on starch digestion properties

It has been stated in botanical studies that the integrity of the cell wall plays an important role in the growth and development of plant. The cell wall integrity helps resists cellular stress and prevents cell rupture.[44] In general, two types of cellular materials are used to study the effects of the cell structure of endosperms on the functional properties of starch in cereals. The first category is intact cells, in which the integrity of the cell wall is maintained (intact cell system, ICS). This category can be further subdivided into two groups, as follows: (1) intact cell clusters (ICC), which are prepared by crushing the grain into tissue fragments (flakes, grits, crude particles, *etc.*) and (2) individual intact cells (IIC), which are separated from starchy tissue using specific methods. The second category is defined as broken cells (refined grain or pulse flour, cereal syrup after homogenization, *etc.*), in which the cell wall integrity is destroyed, and the starch granules contained in the cells are exposed (broken cell system, BCS). In addition, based on specific research questions, two types of research systems are adopted, *i.e.*, model systems (suspension: such as that used in viscosity or swelling power determination) and food systems (bread, pasta, biscuit, porridges, *etc.*).

3.1. Intact cell system

The intact system has been widely reported (Table 2). The nutritional classification of starch into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) is based on the rate and extent of the digestion of starch and its absorption in the small intestine.[4] Early studies on the effect of the cell wall on the digestive properties of starch were mainly conducted using both SDS and RS. These studies aimed to explain the reduced digestibility of starch as an increase in SDS and RS content due to the presence of intact cell walls.[45,46] In other reports, researchers treated samples with cell wall-degrading enzymes and found that the starch digestibility of the samples increased after enzymatic treatment through *in vitro* digestion experiments, which provided a basis for the theory that the cell wall may hinder the digestion of starch.[47] As shown in Fig. 3A and B, Edwards *et al.*[12] performed *in vivo* digestion experiments on wheat endosperm tissue designed as an ICC food system, which revealed that the presence of intact cell wall structures slowed down the digestion rate of starch in endosperm cells. Starch

Table 2 Mechanisms by which the cell wall structure affects starch in intact systems

Raw material systems	Materials	Research systems	Mechanisms	Ref.
ICC	Rice and barley	MS	The protective effect of the cell wall reduces the rate of starch digestion in the raw material and increases the amount of slowly digested starch (SDS).	45
ICC	Grains, pulses, and tubers	MS	The higher content of resistant starch (RS) in pulses is due to the presence of intact cell walls; pressing alters the integrity of the cell wall of the grain, thus increasing the accessibility of starch to amylase and accelerating the rate of digestion.	46
ICC	Barley	MS	The digestibility of starch increased upon treatment with cell wall degrading enzymes.	47
ICC	Durum wheat	FS	Wheat endosperm in farina (2 mm) retained much of its structural integrity during gastric ileal digestion and majorly influenced starch hydrolysis rates and post-prandial metabolism.	12
ICC	Rice	MS	Fluorescence and scanning electron microscopy were used to examine intact samples for any microstructural changes that occurred during *in vitro* digestion. In the intact samples, the endosperm dextrin layer remained as a thin-film during *in vitro* digestion, and therefore may be considered an indigestible substance that affects the overall digestibility of the rice.	48
IIC	Wheat and sorghum	MS	The following conclusions were drawn from microscopic observations of the endosperm cell wall structure, starch, and amylase interactions: (a) wheat and sorghum cell walls are effective barriers to amylase entry; (b) both the extensive protein matrix (especially in sorghum) and the non-catalytic binding of amylase to the cell wall surface can limit amylolytic hydrolysis of starch within intact cells; and (c) the presence of incompletely gelated intact cells in cooked starch suggests limited swelling of granules trapped within the cell wall.	49
ICC	Wheat	MS	The intact cell wall structure limited the expansion of starch; although amylase could pass through the endosperm cell wall, the endosperm cell wall slowed its diffusion and acted as a physical barrier to enzyme diffusion.	50
IIC	Red kidney bean, potato tuber, and banana	MS	The non-catalytic attachment of the cell wall to the amylase enzyme inhibited the utilization of starch by the enzyme and slowed starch digestion.	51
ICC	Peas and green beans	MS	The cell walls of intact cotyledons (*e.g.*, pulses) impede the entry of digestive enzymes and maintain the physical integrity of the cell structure, resulting in the transport of intact cells and their contents from the edible legume to the large intestine.	52
ICC	Maize	MS	The hardness of the endosperm is determined by its intracellular (protein matrix) and extracellular (cell wall) components. Hard endosperm tissue limits the swelling of the starch during pasting, thus reducing its digestibility after cooking.	53
ICC	Barley and rice	MS	Barley with intact cell walls had harder kernels and lower starch digestibility.	54
IIC	Kidney beans	MS	Storage proteins in the cytoplasm acted as a second barrier, preventing the digestion of starch.	56
ICC	Chickpea and durum wheat	MS	The bioaccessibility of starch remained limited in the presence of intact cell walls and the tissue fracture properties and cell wall permeability were the mechanisms by which dietary fiber affected the bioaccessibility of starch.	58
ICC	PulseON®	FS	There was no structural damage to the intact cell walls in PulseON® during the cookie-making process. Replacing 50% of wheat flour with PulseON® reduced the cookie starch hydrolysis index by 60%.	60
ICC	Coarse wheat farina	FS	The addition of coarse wheat farina with its cell structure intact to bread preserved cell wall integrity during production, but the rate and extent of final starch digestion *in vitro* were not altered.	61
ICC	Wheat farina	FS	The addition of wheat farina containing intact endosperm cell did not reduce the starch digestibility in dried noodles.	62
ICC	Large particle size durum wheat flour	FS	The preservation of cell wall integrity reduced the rate of starch digestion in durum wheat flour with larger particle sizes, but it did not act as a barrier to starch digestion in the bread.	63

ICC: intact cell clusters prepared by processing the grain into crushing tissue fragments (flakes, grits, crude particles, *etc.*), IIC: the individual intact cells separated from starchy tissues using specific methods. MS: the model system; and FS: the food system.

granules were still found in the cells toward the center of the endosperm after 4 h of digestion, confirming that the digestibility of wheat starch is affected by the integrity of the cell wall in endosperms. These findings also suggested that controlling the cell wall integrity in endosperms may help control the glycemic response.

In recent years, researchers have explained the mechanisms by which the cell wall affects the digestive properties of starch with the help of microscopic techniques in the ICS and BCS model systems. These mechanisms are summarized in Fig. 4. The influence of the cell wall structure on the characteristics of starch is predominant in the ICS.

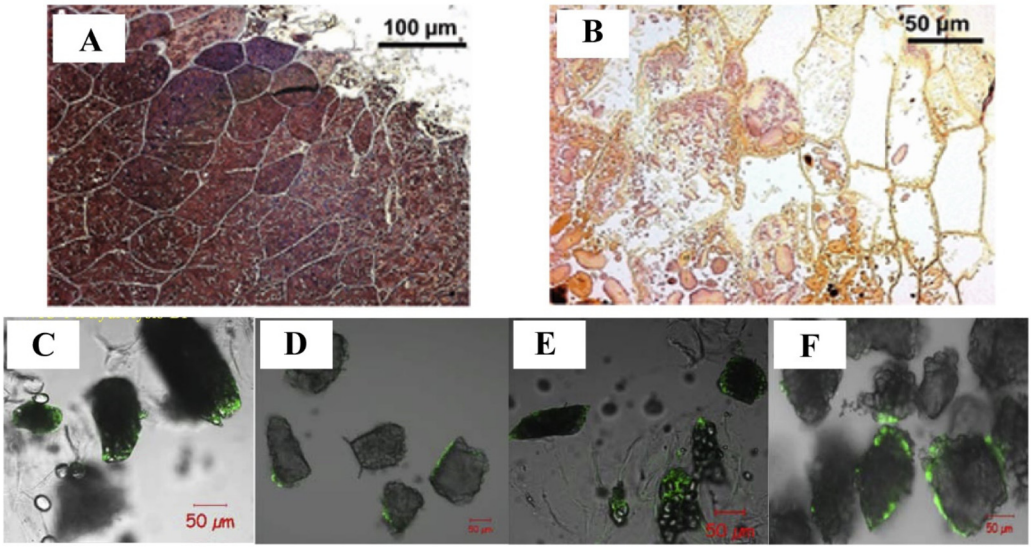

Fig. 3 Light micrographs of undigested wheat endosperm tissue sections (A). High magnification of a typical 2 mm wheat particle found in the ileal effluent 4 h after digestion (B). A and B were stained with 2.5% (wt : v) Lugol's iodine solution and seen under a light microscope. Micrograph of the intact cells of wheat after 1 h of hydrolysis (C). Micrograph of intact cells of sorghum after 1 h od hydrolysis (D). Micrograph of the intact cells of wheat after 4 h of hydrolysis (E). Micrograph of intact cells of sorghum after 4 h of hydrolysis (F). C–F are bright-field images of intact cells and starch (100 mg in 10 mL) using FIT-labeled α-amylase (50 μL; EA 298 CU mL^{-1}) after 1 and 4 h of hydrolysis as seen under a confocal laser scanning microscope. These graphs are modified from Edwards *et al.*[12] and Bhattarai *et al.*[49]

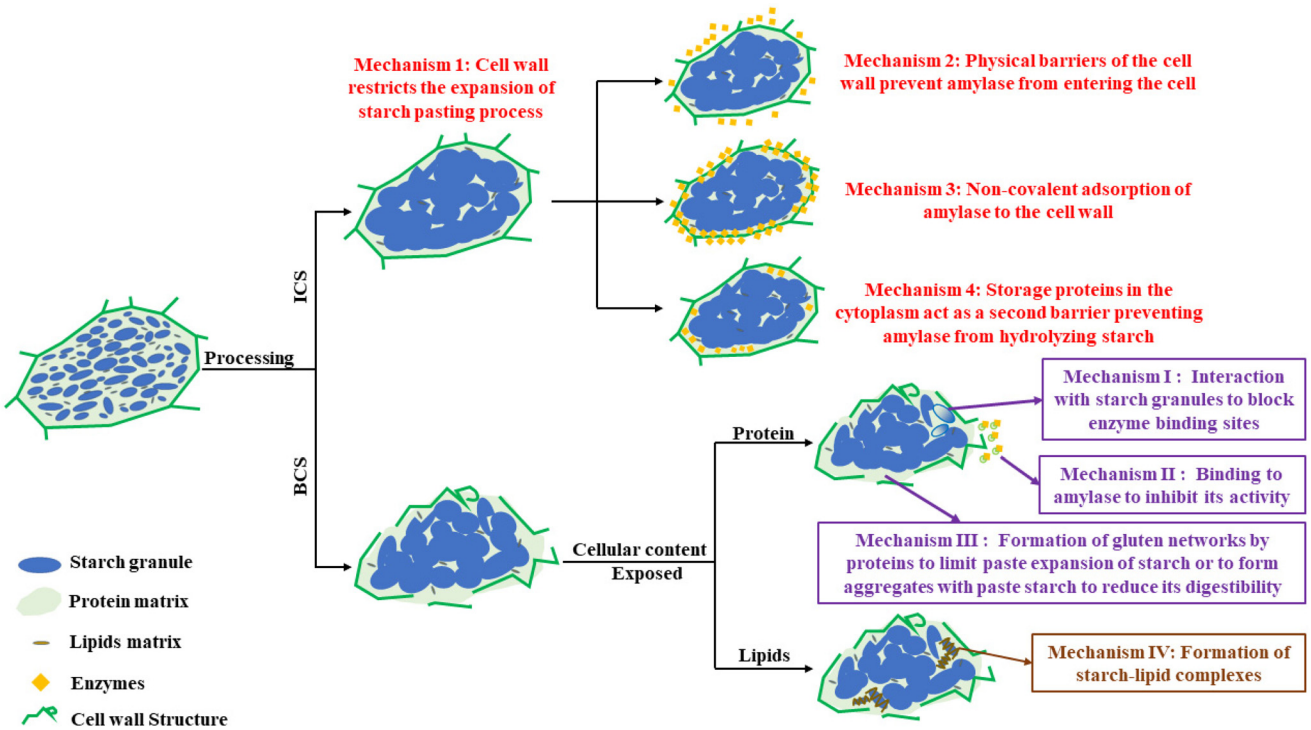

Fig. 4 Mechanisms of influence of cell wall structure on the digestion properties of starch in the intact cell system (ICS) and broken cell system (BCS). The influence of cell wall structure on the digestive characteristics of starch is predominantly visible in ICS and exposed intracellular soluble material is visible in BCS in intact endosperm/cotyledon cells, where starch granules are present in a protein matrix together with a modest quantity of intracellular lipids.

Tamura *et al.*[48] used microscopic techniques and fluorescent labeling to observe the digestion of an intact rice grain (an ICC system) *in vitro* and found that the aleurone layer remained thin and film-like, which hindered the digestion of starch. The cell wall integrity was maintained in the intact tissue structure during digestion. Subsequently, Bhattarai *et al.*[49] reported the interaction among the cell wall structure, starch, and amylase by microscopic observation of individually isolated wheat and sorghum endosperm cells (an IIC system), confirming that the cell wall acts as a barrier to amylase, hindering its entry. Also, both the extensive protein matrix in the intact cells (especially in sorghum) and the non-catalytic binding of amylase on the cell wall surface limited the hydrolysis of starch (Fig. 3C–F). Both studies were confirmed later by other researchers on wheat (type I cell wall) and beans (type II cell wall),[50–52] indicating the prevalence of similar mechanisms in cereals. The intact cell wall structure affects the stiffness of the endosperm, which consequently limits the swelling of starch pastes during cooking. The presence of incompletely gelatinized starch in the cooked intact cells suggests the limited swelling of the granules trapped in the cell wall. These observations were confirmed in previous studies on maize intact tissue (an ICC system),[53] as well as barley and rice.[54]

Previous studies on legume cotyledon cells with their cell wall integrity preserved have shown that they contain partially swollen starch, which becomes gelatinized during cooking. In this case, α-amylase cannot easily penetrate the gelatinized starch granules, thus reducing the rate of starch digestion.[55] Rovalino-Cordova *et al.*[56] carried out digestion experiments using independently isolated cotyledon cells (an IIC system) of kidney beans and observed them under a confocal laser scanning microscope. They found that the storage proteins in the cytoplasm acted as a second barrier, preventing the digestion of starch. This mechanism has been found in monocotyledonous cereals. Yu *et al.*[57] reported that proteins inhibit amylase in barley flour. Edwards *et al.*[58] highlighted the importance of cell wall permeability and tissue fracture properties for starch digestion in chickpea and durum wheat (an ICC system). These studies enriched the research knowledge on the mechanisms by which the cell wall in endosperms can influence the digestive properties of starch. However, further research is needed to apply this mechanism to control starch digestion in cereal or legume products for glycemic control in consumers.

Recently, some research groups made products from cereal/legume flours (an ICC system) with intact cell wall structures to evaluate their starch digestibility in food systems. Edwards *et al.*[59] developed a cell-based bean powder (PulseON®) using isolated individual intact cells as a raw material in 2019. This team used a cell-based powder in a follow-up study to make biscuits with refined wheat flour to show that there was no structural damage to the intact cell walls in PulseON® during the biscuit-making process. Replacing 50% of the wheat flour with PulseON® resulted in a 60% reduction in the starch hydrolysis index of the biscuits.[60] This study demonstrated the feasibility of using legume cell flour to manufacture food products with a low estimated glycemic index (eGI). However, the application of cereal flours with intact cell wall integrity appears to be unfeasible for the production of low eGI foods. Korompokis *et al.*[61] used coarse wheat farina containing intact cell clusters to make bread. They showed that some of the endosperm cell walls in the flour remained intact during bread processing, but the rate and extent of *in vitro* starch digestion were not reduced by the addition of coarse wheat farina. Zhou *et al.*[62] added wheat (*Triticum aestivum* L.) farina containing intact endosperm cells to make dried noodles. They also found that the addition of farina did not significantly reduce the starch digestibility in noodles. Also, Tagliasco *et al.*[63] reported that making bread from durum wheat (*Triticum durum*) flour with large particle sizes presented preserved endosperm cell walls. However, it did not reduce the *in vitro* digestibility of starch in bread, it only affected the digestibility of starch in flour with a large particle size (an ICS system). Therefore, making low-GI foods from grain flour that has preserved cell wall integrity is not a reasonable strategy for cereal processing. In this case, the preservation of the endosperm cell wall integrity during the processing of cereals does not ultimately act as a barrier to starch digestion, whereas it does in legume flour.

Only a few studies have been carried out using cell separation procedures to isolate intact endosperm cells (Table 2). The majority of current research transformed raw materials into powders with intact endosperm cell structures, and then conducted experiments to determine the association between endosperm cells and starch digestion properties. Xiong *et al.*[32] reviewed the basic principles of isolating intact cells in detail, especially from wheat, which is a cereal with a complex seed structure. This may also be the reason why only a few studies have targeted isolated cells. Although microscopic observations and related techniques have been applied at the cellular level using cell powder as a carrier, it remains to be explored whether the other components present in the cellular system (*e.g.*, cell matrix) may cause interference compared to isolated cells. Thus, the differences in the effects of isolated cells *versus* cell powder on starch digestion characteristics warrant further discussion.

3.2. Broken cell system

Endosperm cells contain a large amount of starch and protein and a few lipids encapsulated by the cell wall. In intact endosperm cells, the protein matrix serves as a second barrier for enzymes, water, and heat before they come in contact with starch, reducing the gelatinization and digestibility of starch.[64] Table 3 shows the mechanisms by which the cell contents affect starch digestion in broken cell systems. During processing, cereals are usually finely milled to obtain high-quality products. For example, semi-dried noodles that are made from fine wheat flour have a stronger gluten network.[65] However, fine milling can lead to the breakdown of the endosperm cell wall. When the cell wall is broken during processing, the discontinuous protein bodies inside the cell can be released and interact with each other to form a continuous network, which

increases the overall surface area of cellular materials to interact with starch and affect its properties. Also, when the cell wall is broken, the lipids interact with starch to form a starch-lipid complex, which affects the properties of starch.

The influence of proteins in endosperm cells on the digestive properties of starch is now comprehensively known, and the mechanisms by which the protein matrix interferes with the digestive properties of starch can be summarized as follows: (1) interaction with starch granules to block enzyme binding sites,[66] (2) binding to amylase to inhibit its activity,[67] and (3) formation of gluten networks by proteins to limit the gelation expansion of starch or to form aggregates with gelated starch to reduce its digestibility.[68,69]

The modification of starch with lipids leads to the formation of inclusion complexes called V-type amylose complexes.[70] In general, starch-lipid complexes are more resistant to digestive enzymes than uncomplexed starch.[71] The mechanism of starch-lipid complex formation is based on the helix of glucose residues bound by hydrogen bonds, thus leaving space for guest molecules, such as lipids. In addition, the helical cavity is a hydrophobic tube that readily holds the hydrocarbon chains of lipid molecules in place by van der Waals forces, allowing the polar ends of the lipids to protrude beyond the helical cavity. Conformational changes of amylose from linear to helical have been shown to interfere with the initial stages of starch digestion (enzyme-starch binding) due to the reduced surface area or lack of binding sites.[72–74]

Lipids in endosperm cells interact with starch after cell wall fragmentation, for example, the formation of amylose-lipid complexes leads to changes in the structure of amylose, inhibition of water absorption and swelling of the granules and hydration, pasteurization, and reduced digestibility. Further, the extent of these changes depends on various factors such as chain length and polarity or concentration of lipid ligands.[75] Farooq *et al.*[76] found that 4%–8% of V-type crystalline structures remained in rice after cooking, proving the formation of starch-lipid complexes in the samples, which also led to a decrease in the rate and degree of starch digestion. Shen *et al.*[77] investigated the lipid content of five different varieties of rice and showed that the lipid content was positively correlated with resistant starch content and negatively correlated with the estimated GI and digestibility. Collectively, these observations indicated that the lipids in rice endosperm inhibit the digestion of starch by converting some of the starch into a slowly digestible fraction (starch–lipid complex).

The raw materials selected for the aforementioned study were pasta (*Triticum durum*), rice, corn, sorghum, *etc*., which retained a large particle size after processing. Although these studies were conducted in the broken cell system, based on particle size, it can be inferred that intact cellular structures still existed within the system. In the case of finely milled wheat flour, the cellular structure was not only destroyed, but the structure of starch was lost, leading to changes in its functional properties.[78] There are many reports on the structure–function relationship of starch during milling.[79–82] However, in food processing systems with complete cell fragmentation, the changes in cell structure during processing and their effect on the functional properties of starch remain unclear.

In the broken system, another study focused on the non-starch polysaccharides and phenolic compounds surrounding the cell wall. The non-starch polysaccharides in the cell wall are a class of resistant polysaccharides that cannot be digested, but they also interact with starch and hinder its hydrolysis, thus reducing the rate of starch digestion.[83]

The main polysaccharide in the cell wall of wheat endosperm is arabinoxylan (AX).[84] AX can only be fermented by microorganisms in the colon, where it is degraded into xylan, which has a much lower molecular weight and is beneficial to intestinal health.[85] Rojas-Bonzi *et al.*[86] reported that AX increases the viscosity of bread intestinal digesta, particularly the thickness of the unstirred upper layer on the intestinal mucosal surface, thereby restricting the diffusion of glucose and postponing its absorption by the small intestinal epithelium. AX also interacts with starch, increasing the viscosity of the complex and forming starch-polyphenol complexes, leading to a decrease in the digestion rate of starch.[87] The interaction between the AX extracted from wheat bran and wheat starch has been reported, which indicated that AX with a higher molecular weight leads to a stronger inhibitory effect on starch digestion.[88] However, the exogenous AX and starch in the current study were extracted from cereal bran.[88] Consequently, systematic research on the interaction between AX derived from the endosperm cell wall and starch in the broken cell system is still lacking.

4. The effect of processing modifications on the cell wall structure and digestive properties of starch

Plant-based food materials such as cereals are harvested and subjected to a series of pretreatment steps and extensive processing before they finally become edible foods. These processing operations can affect the cellular structure of plant tissues, for example, pores appear on the surface of the cotyledon cell wall during the soaking of legumes, and the cell wall disintegrates with increasing temperature.[89] Common operations during the processing of cereals and legumes lead to changes in the endosperm/cotyledon cell structure, which influences the digestive properties of starch. In addition, endosperm/cotyledon cells are modified using targeted physicochemical means to give the product specific functional properties, and thus operations introduce modifications to the endosperm or cotyledons at the cellular level.

According to the targeted processing of raw materials that are meant to achieve a specific purpose, cellular modifications can be divided into three main categories (Table 5), as follows: (1) physical modifications, (2) chemical modifications and (3) enzymatic or biotechnological modifications.

Table 3 Mechanisms behind the effect of cellular components on starch digestion properties in broken cell systems

Cellular components	Material	Mechanism	Ref.
Protein	Pasta	The presence of protein hinders the binding of starch to enzymes and makes starch less digestible.	66
	Whole pasta, pasta powder, semolina (with proteins), and extracted starch without proteins.	The protein interacts with α-amylase, leading to a decrease in enzyme activity and slowing down the digestion of starch molecules.	67
	Sorghum and maize	The higher proportion of disulfide bonded cross-linked proproteins and more extensive polymerization of proproteins during cooking form M_r > 100 k polymers, which are responsible for the lower starch digestibility of vitreous sorghum endosperm flour.	68
	Pasta	The starch granules in the central region of the cooked pasta are not completely gelated due to the compact structure of the gluten network that limits the expansion of the starch granules. The competition between the gluten network and the starch granules for water prevents the starch granules from obtaining water, thus slowing down their digestion.	69
Lipids	Rice	The digestibility of rice decreases significantly with increasing particle size. The digestibility of non-waxy rice flour was lower than that of waxy rice flour, probably due to the formation of starch–lipid complexes.	76
	Rice	Rice endosperm lipids inhibit starch digestion by converting some of the starch into a slowly digestible starch fraction.	77
Arabinoxylan (AX)	Wheat bread	AX increases the viscosity of the bread intestinal digesta and the thickness of the unstirred layer on the intestinal mucosal surface. This results in the restriction of the diffusion of glucose and postpones its absorption by the small intestinal epithelium.	86

4.1. Physical modifications

In the primary processing stage of raw grains, grains are usually milled to make flour with a specific particle size, which is then converted into grain products by additional operations, such as dough mixing, fermentation, and rolling. Finally, the flour is transformed into edible grain products by steaming and baking.[90] All these processing methods introduce physical modifications to the endosperm/cotyledon cells at the cellular level. RamyaBai *et al.*[91] evaluated the GI of four cereal products in this regard, where the effect of milling on the cereal endosperm was explored by microscopic techniques, confirming that milling disrupts the structural integrity of the endosperm. However, the integrity of the endosperm or cotyledon cells can be maintained if the size of the final product after processing is accurately controlled during milling. As mentioned in section 2, the size of cereal endosperm cells and legume cotyledon cells is between 80–120 μm. Theoretically, controlling the size of the flour particles above the cell particle size during milling can result in the formation of flour containing complete cell clusters. The relationship between the particle size of flour and cell integrity was explored in some reports (Table 4), which showed that the closer the particle size in the final milled product and the cell size, the more susceptible the cells to disruption. As mentioned previously, if the endosperm/cotyledon cell integrity is disrupted, the digestion properties of starch are affected in a series of ways in the broken cell system.

In addition to milling, the effects of baking, heat fluidization, and heat-moisture treatment (HMT) on endosperm/cotyledon cells have been reported. Comino *et al.*[92] reported the effect of typical food processing conditions (dough formation, baking, extrusion, and steaming/boiling) on the structure and extractability of dietary fiber from rye, hull-less barley, and

Table 4 Relationship between grind size and cell integrity

Raw material	Endosperm/cotyledon cell size/μm	Flour fractions	The particle size of flour/μm	The integrity of endosperm/cotyledons	Ref.
Black wheat	200 (L) × 50 (W)	D_{50}	90	Partial preservation of cellular integrity	113
Maize	120 (L) × 70 (W)	Flour	85 ± 5	Cellular integrity is disrupted	50 and 64
		Fine farina	330 ± 5	Partial preservation of cellular integrity	
		Coarse farina	705 ± 5	Cellular integrity is retained to a large extent	
Navy bean	50–100	Small	<74	Cellular integrity is disrupted	17 and 114
		Medium	74–297	Cellular integrity is retained to a large extent	
		Large	297–500	Cellular integrity is retained to a large extent	

When the particle size of the material follows a normal distribution, the volume-median diameter (D_{50}), which is measured by a laser particle sizer, may be representative of the characteristics of the sample size.

Table 5 Effect of endosperm cell modification on the digestive properties of starch

Modification type	Material	Modification methods	Effects and mechanisms of modification on endosperm cells	Ref.
Physical modification	Kashi 7 whole grain pilaf (WGP), instant brown rice (IBR), refined maize ugali (RMU), and whole maize ugali (WMU)	Milling	Microstructural inspection of the IBR showed a disrupted bran layer with cracks, indicating a loss of bran integrity. Stereoscopic images of the WGP showed intact bran and germ. For the RMU and WMU, the grains were crushed, resulting in a loss of integrity.	91
	Endosperm flour of wheat, rye, and barley	Dough formation, baking, extrusion, and steaming/boiling	The cell walls were gradually disrupted and the loose β-glucan was dispersed. At least some arabinoxylan appeared to be more anchored within the cell walls. The total amount of AX and β-glucan was not significantly affected by processing, but the soluble fiber fraction of each type of flour showed a similar increase during baking, extrusion, and cooking.	92
	Popcorn maize and sorghum	Popped with hot-air	*In vitro* digestibility of treated sorghum was much lower, suggesting that bursting-induced fragmentation of the sorghum wall improved the accessibility of protein and starch reserves in the endosperm to the digestive enzymes.	93
	Highland barley	Heat fluidization, microwave roasting, and baking	After heat treatment, the formation of cracks was observed appearance. The microstructural images showed that a large number of micropores were uniformly distributed in the endosperm structure and the cells of the wheat endosperm layer were compressed and deformed. In addition, significant disruption of the endosperm cell wall and slight deformation of the outer layer were observed by confocal laser scanning microscopy.	94
	Highland barley	Heat fluidization, microwave roasting, and baking	The integrity of the endosperm cells remaining in highland barley flour after hot fluidization, microwave roasting, and baking was reduced to different degrees, where hot fluidization caused the most significant damage to the cell integrity, baking the second, and microwave roasting the least.	95
	Pinto bean	Heat-moisture treatment (HMT)	HMT did not disrupt the integrity of the cell wall of pinto bean cotyledons. The digestion rate of starch in intact cotyledon cells was slower after HMT treatment compared to isolated starch.	96
	Garbanzo and pinto bean	Hydrothermal treatment	The integrity of the cotyledon cell wall was not damaged in the range of 70–100 °C. The cotyledon cell wall always acted as a barrier to the digestion of starch granules within the cotyledons, reducing the digestibility of starch.	97
	Chickpea	Gaseous ozone	In the range of 500–1000 ppm, the damage to the cell wall increased with increasing ozone concentration and the integrity decreases. At 1000 ppm, the cell wall broke down and the starch granules were dispersed outside the cell wall	98
Chemical modification	Sorghum	Base (NaOH) treatment	It is recognized that soaking grains in 0.2% NaOH can increase grain hydration by loosening the endosperm cell walls.	99
	Rice	Oil-frying and popping	Cross-linked starch structures were produced in the rice kernels by citric acid treatment. This structure greatly increased the tensile strength by strengthening intermolecular interactions. Frying or popping at high temperatures and pressures can be used to process rice or other grains to gelatinize the starch molecules without water.	100
	Intact cells isolating from potato tubers	Chemically cross-linked with sodium trimetaphosphate/ sodium tripolyphosphate	Both starch and cell wall polysaccharides were cross-linked, and the swelling power of starch decreased significantly with increasing degrees of cross-linking. The cross-linking reaction significantly reduced the rate and extent of starch digestion in both intact and disrupted cells after cooking. In addition, the barrier effect of the cell wall on starch digestion increased with the degree of cross-linking because limited starch swelling and pectin dissolution during cooking partially protected the integrity of the cell wall, thereby decreasing its permeability.	101
Enzymatic or biotechnological modification	Wheat	The enzyme (ferulic acid esterase) and base (NaOH) treatment	Wheat endosperm cell walls lose their structural integrity after enzyme/alkali treatment and release more polysaccharides into the solution. The breakage of ferulic acid crosslinks could be used to modify the rheological behavior of cell wall polysaccharides in wheat flour, with implications for both nutritional and processing functions.	103
	Pigeon pea and horse gram seeds	Inorganic salt ($NaHCO_3$) and protease	Sodium bicarbonate and protease pretreatment altered the cell wall structure of these legumes, leading to the production of puffed grains. The increase in porosity and decrease in cell wall thickness in the puffed grains led to the collapse of the cell walls and the appearance of large voids in the intercellular matrix.	105

wheat endosperm flours. They showed that these operations result in the progressive disruption of endosperm cells and the partial dissolution of the non-starch polysaccharides that are physically bound to the cell wall. Parker *et al.*[93] investigated the structure of vitreous endosperm of raw and popped kernels of corn and sorghum by microscopic techniques, which demonstrated that the cell wall of vitreous endosperm breaks into tiny fragments during the explosion. It was also observed that in the cells of vitreous endosperm, the heated starch granules had the potential to swell without fragmenting the cell wall. Although the authors only evaluated the protein accessibility, it can be inferred from this and other available studies that popping of cotyledons or endosperms with hot-air modification may not accelerate starch digestion given that the cell wall junction integrity is not disrupted. However, whether this type of cell modification changes the digestive properties of starch still needs further studies. Bai *et al.*[94] treated highland barley with heat fluidization, microwave roasting, and baking, which led to the formation of cracks in the endosperm, and microscopic observation showed that a large number of micropores was uniformly distributed in the endosperm structure. Furthermore, significant disruption of the endosperm cell wall and deformation of the outer layer were also observed by confocal laser scanning microscopy. Bai *et al.*[95] investigated the treated highland barley flour in a subsequent study using fluorescence microscopy and reported that endosperm cells with an intact cell wall structure were observed in the untreated highland barley flour, which had a size of ~100 μm. The size of cellular fragments remaining in the highland barley flour after thermal fluidization and baking was <50 μm and ~50 μm, while that retained after microwave roasting was ~100 μm. Thus, the integrity of the highland barley endosperm cell wall may not have been destroyed after microwave roasting, and the internal starch could remain encapsulated by the cell wall. However, the other possible changes to the digestive properties of starch as a result of this process still need further experimentation. Xiong *et al.*[96] treated intact cotyledon cells isolated from a pinto bean with HMT at 100 °C and 15%, 25%, and 35% water content for 8 h. The digestion rate of starch in the cotyledon cells that retained their cell wall integrity was lower than that of the isolated starch, indicating that the passage of amylase was still hindered by the cell wall structure after HMT. Therefore, HMT did not disrupt the integrity of the cotyledon cells. Li *et al.*[97] isolated intact cotyledon cells and starch granules from garbanzo and pinto beans and subjected the isolated cells and isolated starch to hydrothermal treatment at 70 °C, 80 °C, 90 °C, and 100 °C. The integrity of the cotyledon cell wall was not damaged at any of these temperatures, allowing it to act as a physical barrier to intracellular starch and reduce its digestibility. The authors drew these conclusions by observing the cellular structure and enzyme sensitivity as well as the digestibility of starch at different treatment levels. Nickhil *et al.*[98] evaluated the effect of gaseous ozone on the microstructure of chickpea granules at 500–1000 ppm of ozone. At 500 ppm, the distribution of starch granules in the chickpea cotyledon cells changed, suggesting that ozone may lead to the hydrolysis of α-glycosidic bonds, loosening the starch granules. At 750 ppm, the cotyledon cells were further deformed, but the cell wall was not damaged and the shape of the starch granules did not change. At 1000 ppm, the cotyledon cell wall showed an irregular shape and a large number of starch granules appeared on the surface of the cell wall, but the starch granules were not damaged, indicating that the cell wall integrity was compromised at this very high ozone concentration.

In conclusion, for the same raw material, different methods of inducing physical modification can have different effects on the integrity of the cell wall. The same physical modification method can also have different effects on the cell wall integrity depending on the level of variation. In these studies, physical modification did not tend to modify the starch granules inside the endosperm/cotyledon cells if the cell wall integrity was intact. Alternatively, structural damage caused by the physical modification of the endosperm/cotyledon cell wall resulted in changes in starch digestibility.

4.2. Chemical modifications

During the processing of cereal products, the extraction or pretreatment of the raw material is carried out with acids, bases, or enzymes, which can be considered as chemical modification or enzymatic/biotechnological modification of the endosperm/cotyledon cells.

Tawaba *et al.*[99] treated sorghum using a base (NaOH), showing that soaking seeds in 0.2% NaOH improved the hydration of the grain by opening the endosperm cell wall. However, whether the effect of this alkali treatment on the endosperm cell wall permeability also induced changes in amylase permeability deserves further investigation. Lee *et al.*[100] used citric acid to treat rice, and then cooked it using deep-frying and blasting processing. They observed that citric acid affected the cross-linking of the starch molecules and led to lower blood glucose in the consumer. Therefore, deep-frying or blasting under high temperature and pressure conditions can be used to process rice or other cereals to achieve gelatinization of starch molecules without water and slow down their digestion.

In the above-mentioned studies, treatment with acid or base modified the cell wall but did not change the properties of the intracellular starch. In contrast, in studies on plant foods rich in starch except for cereals, the chemical modification treatments caused changes in both the cell wall structure and starch properties.

Ding *et al.*[101] treated intact cells isolated from potato tubers. Specifically, they sorted and chemically cross-linked starch in the tubers using phosphate, showing that not only phosphate cross-linking the cell wall polysaccharides, but also the cross-linking significantly reduced the rate and extent of starch digestion in both intact and disrupted cells after steaming. In addition, the barrier effect of the cell wall on starch digestion increased with the degree of cross-linking. This was because the limited starch swelling and pectin dissolution during cooking partially protected the integrity of the cell wall, thereby reducing its permeability. Partial modification not only led to modification of the cell structure, but it also

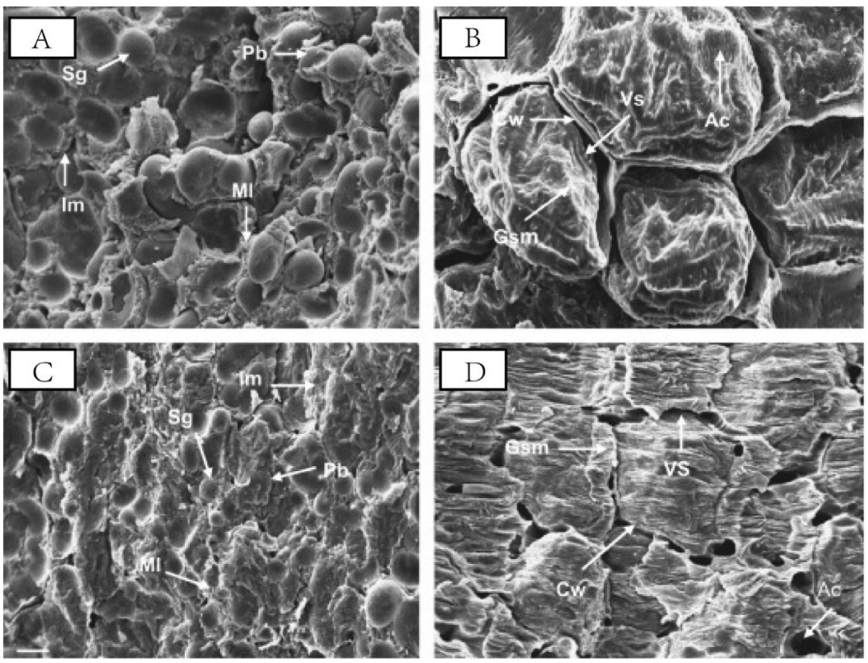

Fig. 5 Scanning electron micrograph sections of pigeon pea and horse gram. (A) Pigeon pea before magnification and (B) after magnification. (C) Horse gram before magnification and (D) after magnification. Gsm: gelatinized starch matrix; Pb: protein bodies; Cw: cell wall; Sg: starch granules; Ml: middle lamella; IM: intercellular matrix; Ac: small air cells; and Vs: void spaces. The scale bar is 10 μm for (A–D). These graphs are modified from Sreerama *et al.*[105]

directly affected the starch granule. Although the above-mentioned report[101] is a study conducted on tuber plants and there is a lack of research on is the occurrence of similar chemical cross-linking modifications in cereals, it provides a new idea for the study of potential modifications in cereal endosperm cells.

In summary, the effect of the chemical modification of endosperm cells on the digestive properties of starch is mainly reflected in whether the cell integrity is disrupted and the permeability of the cell wall is altered. Studies on several plant food tissue cells have demonstrated that the chemical modification of cells is accompanied by a change in the properties of starch, where the starch granules are also affected by the modification. This is attributed to the fact that the size of the pores in the cell wall is generally larger than the molecular size of the modifier, which allows it to enter the cell and interact with the starch granules.[102]

4.3. Enzymatic or biotechnological modifications

For enzymatic or biotechnological modification, Gartaula *et al.*[103] isolated intact endosperm cells from flour and cleaved the ferulic acid dimers in the cell wall using ferulic acid esterase and sodium hydroxide. The enzyme/base treatment caused the cell wall to lose its structural integrity and the ferulic acid cross-linkage was broken, resulting in a change in the rheological properties of the wheat endosperm, which originally had a semi-solid to liquid-like nature. When the viscosity of the endosperm cell mixture was reduced, its rate of digestion in the small intestine became faster with lower viscosity foods.[104] Sreerama *et al.*[105] performed multiple modifications of legume species, pigeon peas, and horse gram seeds by pretreating them with $NaHCO_3$ and protease. This treatment changed the cell wall structure in the bean cotyledons, producing expanded grains with increased porosity, which decreased the thickness of the cell wall and caused it to collapse, eventually leading to the appearance of a large number of pores in the intercellular matrix (Fig. 5). As shown in Fig. 5, starch gelatinization occurred in the expanded grains after treatment. Combined with changes in the cell wall after modification, it can be inferred that the digestibility of starch may increase after modification.

In conclusion, enzymatic modification has a substantive effect on the cell wall structure. The buffer solution for enzymes during enzymatic modification often contains inorganic acids, bases, or salts, and thus it may act as a chemical modifier capable of changing the structural and functional properties of starch granules. Thus, enzymatic modification is similar to chemical modification in that the effects of both modifications on the digestive properties of starch are also reflected in altered cell wall integrity and structural and functional properties of starch.

5. Conclusions and future remarks

In this review, we summarized the current research on intact and broken cell systems. In addition, we summarized the biophysical properties of endosperm/cotyledon cells of common cereals and legumes. The modification of the endosperm/coty-

ledon cell structure during the processing of cereals and legumes was also reviewed. This information will enable researchers to better understand the relationship between the structure of endosperm/cotyledon cells and the digestive characteristics of starch. According to the discussion herein, it was noted that there are still some pressing issues in this field that must be addressed. Firstly, although the cell wall integrity can be preserved in foods, whether it continues to act as a barrier to fast starch digestion depends on the processing intensity of cereals and legumes. If cereals and legumes that have undergone mild treatment are processed into food or food ingredients (*e.g.*, porridge and flour with large particle size), the digestibility of starch in these products is reduced and has a lower GI value. However, if cereals and legumes are processed into food following strong treatment, such as in bread, the cell wall no longer acts as a barrier and the digestibility of starch is no longer significantly different from that of a normal product and cannot have a lower GI value. Therefore, producing cereal and legume products (with strong treatment) with an intact cell structure is not a feasible approach given that it results in a low GI. Secondly, aspects that need further systematic study include a better understanding of the structural changes in cereals and legumes caused by modification in the cell structure with different physicochemical and biological means during processing and the effect of the modification conditions on the physicochemical properties of starch when the cell wall integrity is preserved. Thirdly, other understudied aspects include the effect of cell wall-derived substances on the digestive properties of starch, where undesirable materials may be released as cereals and legumes are strongly processed and their endosperm/cotyledon cell structure is destroyed in the crushing system.

In conclusion, future research should focus on revealing the patterns of changes in the structure of endosperm/cotyledon cells during the processing of cereals and legumes and downstream product preparation. The mechanisms by which the cell wall-derived components affect the functional properties of starch in endosperm/cotyledons during cell structure fragmentation should also be further discussed. A better understanding of these mechanisms is needed to help develop more functional grain and legume products with low GI values.

Author contributions

Jiasheng Wang: writing – original draft, formal analysis, investigation, data curation, writing – review & editing. Chong Liu: conceptualization, supervision, resources, project administration, funding acquisition, writing – review & editing. Xueling Zheng: resources, funding acquisition. Jing Hong: writing – review & editing. Binghua Sun: writing – review & editing. Mei Liu: writing – review & editing.

Conflicts of interest

There are no financial interests or personal relationships that may affect the competing work of this report.

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