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Granule Size Heterogeneity in Barley Starch: Structural, Compositional, and Functional Differences between A- and B-type Granules and Their Industrial Implications

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Abstract

Barley (Hordeum vulgare L.) is unique among cereal crops for the way its starch is organized not as a uniform population of granules, but as two structurally and functionally distinct classes. Large lenticular A-type granules (10–30 μm) and small, irregularly shaped B-type granules (≤6 μm) coexist in a bimodal size distribution that is rarely observed in other cereals. Numerically, B-type granules dominate (80–90% of total count), yet A-type granules account for 85–90% of total starch mass. This asymmetry is far more than a morphological curiosity: it reflects deep-seated differences in amylose content, lipid-complexed amylose concentrations, crystalline architecture, and surface porosity that collectively govern how barley starch behaves from the farm gate through to food processing, brewing, and bioethanol production. This review provides a comprehensive synthesis of current knowledge on the structural and functional differences between A- and B-type barley starch granules, considering three genotype backgrounds: normal, waxy, and high-amylose. The evidence consistently shows that B-type granules possess higher gelatinization temperatures, lower gelatinization enthalpies, greater enzyme hydrolysis rates under mild conditions, higher peak viscosities, and weaker gel network structures compared to A-type granules. Importantly, some properties, including swelling factor in high-amylose genotypes and setback viscosity, reverse direction depending on the genotype, underscoring the complex interplay between granule size and genetic background. The review also identifies significant research gaps in granule-size-specific chemical modification, freeze-thaw stability, and multi-scale structural characterization, and discusses how these gaps limit the full industrial exploitation of barley starch's inherent heterogeneity.

Keywords: Barley starch, A-type granules, B-type granules, Bimodal size distribution, Gelatinization, Pasting properties, Enzyme hydrolysis.

1 | Introduction

Barley (Hordeum vulgare L.) has sustained human civilizations for thousands of years, and yet its biochemistry continues to surprise. As the fourth most cultivated cereal crop globally, trailing only wheat, maize, and rice, barley has traditionally been associated with malting and brewing. More recently, however, its starch has

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attracted growing scientific and commercial interest, largely because of a structural feature that sets it apart from nearly all other cereal starches: A pronounced bimodal granule size distribution.

Unlike maize, rice, or potato starch, which produce relatively uniform granule populations, barley starch exists in two clearly defined classes. Large, lens-shaped A-type granules (10–30 μm in diameter) form early in grain development and ultimately account for the bulk of starch mass, while tiny, irregular B-type granules (≤6 μm) form considerably later and vastly outnumber their larger counterparts. This bimodal architecture is shared by other members of the Triticeae tribe, wheat, rye, and triticale, but is absent in most other commercially important cereals.

The practical significance of this size duality is considerable. During malting, A-type granules gelatinize and are hydrolyzed within minutes at standard mashing temperatures, while B-type granules may persist intact well beyond those temperatures, contributing to dextrin formation rather than fermentable sugar production. In food processing, the two populations behave differently during heating, gel formation, and storage. And from a nutritional standpoint, the higher crystallinity and lipid content of B-type granules raise intriguing questions about their potential contribution to resistant starch formation and glycemic response.

Despite these well-known distinctions, the majority of published research has characterized bulk (mixed) barley starch rather than isolated granule fractions. This imbalance is largely a practical limitation; fractionating A- and B-type granules at sufficient purity and yield remains technically demanding, but it means that the field's understanding of size-specific behavior remains incomplete, particularly across the three major genotypic backgrounds: normal, waxy, and high-amylose.

This review brings together the available evidence on A- and B-type barley starch granule properties, drawing on decades of foundational work alongside recent advances in hull-less barley characterization, multi-scale structural analysis, and processing science. Our aim is not only to synthesize what is known, but to identify the specific knowledge gaps that, if addressed, could enable more rational and targeted use of barley starch across food, feed, and industrial sectors.

2 | Barley Grain Structure and Starch Biosynthesis

2.1 | Kernel Architecture and Starch Localization

The barley kernel is architecturally layered. The endosperm, contributing roughly 75–80% of total kernel weight, is where starch is synthesized and deposited. Within this tissue, starch granules are embedded within a protein matrix, surrounded by cell walls rich in β-glucan (70–75%), arabinoxylans (20–25%), and protein (5–6%). The outermost endosperm layer, the aleurone, contributes 5–10% of kernel weight and has a notably different composition, dominated by arabinoxylans (60%), β-glucan (22%), and proteins (16%).

Hull-less barley varieties developed to eliminate the outer fibrous hull have attracted considerable interest in recent decades because they retain more nutritional components and eliminate the need for pearling before processing. Compared to their hulled counterparts, hull-less varieties contain more starch on a dry weight basis (approximately 59.7% vs. 53.7%) and more lignin, while hulled barleys show higher dietary fiber (18.6% vs. 13.8%) and ash content.

2.2 | Developmental Timing of Granule Formation

One of the most important and often underappreciated facts about A- and B-type granules is that they do not form at the same time. A-type granule synthesis begins approximately 4–5 days after anthesis (flowering), during the early grain-filling phase when endosperm cell division is most active. Their growth ceases after this cell division stage concludes. B-type granules, by contrast, do not begin forming until approximately 16–19 days after anthesis, and they continue accumulating throughout the remainder of grain development.

This temporal separation matters enormously for understanding compositional differences. A-type granules form under different metabolic conditions, different enzyme concentrations, substrate availability, and hormonal milieu than B-type granules, which emerge later in a more lipid-rich endosperm environment. Consequently, B-type granules accumulate more internal lipid and develop a more tightly organized crystalline architecture, even though they form after their larger counterparts.

2.3 | Fractionation Approaches

Separating A- and B-type granules with sufficient purity for functional analysis has been achieved primarily through two routes: gravitational sedimentation (decantation) and pin-milling followed by air classification. Vasanthan and Bhatty (1995) showed that pin-milling and air classification of hull-less barley produced a starch-rich fraction (77.78% starch) enriched in large A-granules, with a final starch yield of approximately 34%. More recent work has employed gravitational field-flow fractionation coupled with UV/VIS detection to achieve separation purities exceeding 95% for each fraction, a significant technical advance that enables more reliable structure-function studies.

3 | Granule Morphology, Ultrastructure, and Crystallinity

3.1 | Size, Shape, and Surface Architecture

Viewed under scanning electron microscopy, A-type barley starch granules present as classic biconvex lens shapes or broad, disc-like structures typically 10–30 μm in diameter. B-type granules, by comparison, are considerably smaller (≤6 μm) and lack this regular morphology, appearing spherical or polyhedral and often with irregular surface contours. The four-to-five-fold difference in linear dimension translates to an approximately 64–125-fold difference in granule volume, with dramatic consequences for surface area-to-volume ratio.

Surface pores are present in both granule types in normal and high-amylose genotypes, with diameters reaching up to 0.9 μm in the equatorial groove region. These pores are not merely structural features; they are functionally important entry points for water, enzymes, and chemical reagents. Transmission electron microscopy has revealed internal channels extending inward from these surface pores in waxy and normal genotypes, lined with proteins and phospholipids that form a membrane-like structure regulating permeability. High-amylose genotypes show less consistent channel development, suggesting that the very high amylose content in those varieties alters granule interior organization.

Table 1. Morphological and structural characteristics of A- and B-type barley starch granules.

Property	A-type Granules	B-type Granules	Genotype	Reference
Shape	Large lenticular/disc-shaped	Small, irregular/spherical	All genotypes	Vasanthan and Bhatty, [1].
Size range	10–30 μm	≤6 μm	All genotypes	Li et al., [2].
% by number	10–20%	80–90%	Normal	MacGregor and Fincher, [3].
% by weight	85–90%	10–15%	Normal	MacGregor and Fincher, [3].
Surface pores	Present (≤0.9 μm)	Present	Normal, High-amylose	Li et al., [2].
Internal channels	Present (waxy, normal)	Present (waxy, normal)	Waxy, Normal	Li et al., [2].
Biosynthesis onset	4–5 days after anthesis	16–19 days after anthesis	All genotypes	Ao and Jane, [4].

Note: Morphological data compiled from multiple studies. Pore diameters refer to equatorial groove region. Internal channel composition (proteins + phospholipids) reported primarily for hull-less waxy barley.

3.2|X-ray Diffraction and Relative Crystallinity

Both granule types display the A-type X-ray diffraction pattern characteristic of cereal starches, with characteristic reflections at 2θ values of approximately 15°, 17°, 18°, and 23°. Some barley cultivars with intermediate amylose contents can exhibit a mixed A+B type pattern, reflecting disrupted packing in the crystalline lamellae. An important and somewhat counterintuitive finding is that A-type granules consistently show higher Relative Crystallinity (RC) than B-type granules from the same genotype, yet B-type granules gelatinize at higher temperatures. This apparent paradox is resolved when one considers the higher lipid-complexed amylose content of B-type granules, which restricts water uptake and crystallite disruption during heating independently of bulk crystallinity. The V-amylose–lipid complex peak at approximately 20° 2θ is detectable in both granule types, including in waxy barley starch, a reminder that even very low levels of amylose can form stable lipid complexes.

3.3|Lamellar and Nanostructure

Small-angle X-ray scattering studies on hull-less barley have demonstrated that B-type granules have thicker semi-crystalline lamellae and a higher ratio of crystalline to amorphous regions than A-type granules. This more ordered nanostructure in B-granules, combined with their higher lipid-complexed amylose content and the prevalence of short amylopectin fa-chains (DP 6–12), creates a densely packed interior that resists both water ingress during gelatinization and enzyme penetration during hydrolysis.

4|Chemical Composition of A- and B-type Granules

4.1|Amylose and Amylopectin

In normal barley, A-type granules contain approximately 28.1% amylose compared to 23.0% in B-type granules, a relationship that is actually the reverse of what is observed in wheat, where B-granules typically carry more amylose. This distinction matters because amylose content strongly influences gelatinization, retrogradation, and enzyme hydrolysis behavior. In high-amylose genotypes, the relationship between granule size and amylose content becomes more complex and may reverse, driven by the overwhelming dominance of amylose biosynthesis throughout the entire endosperm.

Amylopectin chain length distribution also differs between the two populations. A-type granules show a higher average degree of polymerization (DP = 26.7 units) than B-type granules (DP = 24.9 units). B-type granules contain a higher proportion of short-chain fractions (fa-chains, DP 6–12), which supports a more densely packed lamellar arrangement and contributes to their greater crystalline order at the nanoscale.

4.2|Lipids and Lipid-Complexed Amylose

If there is a single compositional variable that most comprehensively explains the functional differences between A- and B-type barley starch granules, it is lipid content, particularly internal lipids in the form of lysophospholipids. B-type granules consistently contain higher internal lipid concentrations than A-type granules, and this difference is most pronounced in high-amylose genotypes. In high-amylose barley, lipid-complexed amylose (L·AM) reaches 12.1% in small B-type granules compared to just 4.1% in large A-type granules, a three-fold difference with consequences for virtually every functional property measured.

Lipid-amylose inclusion complexes physically crosslink amylose chains, restrict granule swelling, suppress Amylose Leaching (AML), reduce gelatinization enthalpy (because complexed amylose does not contribute to the main DSC endotherm), and create a barrier to enzymatic attack. Understanding L·AM content is therefore not merely an academic exercise but a practical predictor of how a given granule population will behave during cooking, fermentation, and digestion.

Table 2. Chemical composition of A- and B-type barley starch granules across genotypes.

Component	A-type Granules	B-type Granules	Genotype	Reference
Amylose content (%)	28.1% (normal)	23.0% (normal)	Normal	Devi et al. [5]
Amylopectin DP (CL)	26.7 units	24.9 units	Normal	Obadi et al. [6]
Internal lipid (%)	3.2–4.1% (high-amylose)	7.1–12.1% (high-amylose)	High-amylose	Vasanthan and Bhatty [1]
Surface lipid (%)	0.1–0.2%	0.1–0.2%	All genotypes	Li et al. [2]
L·AM-waxy (%)	Lower	Higher	Waxy	Morrison et al. [7]
Rel. Crystallinity-waxy	44.6%	38.9%	Waxy (SB89528)	Vasanthan and Bhatty [1]
Rel. Crystallinity-normal	36.8%	33.5%	Normal (Condor)	Vasanthan and Bhatty [1]
Rel. Crystallinity-high-amylose	41.8%	37.2%	High-amylose (Glacier)	Vasanthan and Bhatty [1]

Note: DP = degree of polymerization; L·AM = lipid-complexed amylose. Values represent ranges across cultivars. Higher crystallinity in A-type granules does not translate to higher gelatinization temperature due to the dominant effect of L·AM in B-type granules.

5 | Gelatinization Properties

5.1 | Thermal Characteristics: DSC Analysis

Gelatinization, the irreversible transition from ordered granule structure to a disordered paste, is one of the most studied and functionally critical properties of starch. When barley starch granules are heated in excess water, the disruption of hydrogen bonds between starch chains allows water to penetrate, granules swell, birefringence is lost, and amylose leaches into the surrounding medium. Differential Scanning Calorimetry (DSC) has been the workhorse method for characterizing this transition in size-fractionated granule populations.

The most consistent finding across the literature is that B-type granules exhibit a wider gelatinization temperature range (Tc–To) than A-type granules in all three genotypes. For normal starch, this range is 16°C for small B-granules versus 13.5°C for large A-granules; for waxy starch, 16°C versus 13°C; and for high-amylose starch, 21.5°C versus 18°C. Peak gelatinization temperature (Tp) is also consistently higher for B-type granules, a result that appears paradoxical given their lower RC, but is well explained by their higher content of thermally stable lipid-complexed amylose.

Gelatinization enthalpy (ΔH) tells the opposite story: B-type granules consistently show lower ΔH than A-type granules, despite their higher Tp. This observation is because L·AM complexes melt at a separate, higher temperature range (approximately 95–105°C) and therefore do not contribute energy to the main DSC gelatinization peak. The net result is that B-type granules are harder to start gelatinizing, but require less total energy once the process is initiated.

Table 3. Gelatinization parameters of isolated A- and B-type granules from normal, waxy, and high-amylose barley starches.

Starch / Granule	To (°C)	Tp (°C)	Tc (°C)	Tc–To (°C)	ΔH (J/g)	Reference
Normal-Small (B)	54	61	70	16	6.8	Vasanthan & Bhatty, [1]
Normal-Large (A)	54	58	67.5	13.5	7.9	Vasanthan & Bhatty, [1].
Waxy-Small (B)	60	64	76	16	9.9	Vasanthan & Bhatty, [1].
Waxy-Large (A)	59	62.6	72	13	11.4	Vasanthan & Bhatty, [1].
High-amylose-Small (B)	58	68.4	79.5	21.5	6.5	Vasanthan & Bhatty,[1].
High-amylose-Large (A)	59.3	65.7	77.3	18	7.8	Vasanthan & Bhatty [1].
Malting varieties (range)	56.5–58.5	61.2–63.0	66.7–68.7	—	6.49–9.61	Pycia et al. [8].

Note: To = onset temperature; Tp = peak temperature; Tc = conclusion temperature; ΔH = gelatinization enthalpy. Starch: water ratio = 1:3 unless otherwise noted.

6 | Swelling Factor and Amylose Leaching

When starch granules are heated beyond their gelatinization temperature, water molecules penetrate the disrupted crystalline regions, and the granule swells. Swelling Factor (SF) quantifies the volume increase relative to the dry granule state, while AML tracks the diffusion of amylose chains out of the swelling granule into the surrounding water.

6.1 | The High-Amylose Reversal

In waxy and normal barley starches, small B-type granules swell more than large A-type granules across the temperature range of 50–95°C, an intuitive result, since their higher surface area-to-volume ratio facilitates water uptake. In high-amylose starch, however, this relationship reverses completely: B-type granules show lower swelling than A-type granules. This reversal is one of the most intriguing paradoxes in barley starch science.

The reversal in high-amylose starch is best explained by the unusually high concentration of lipid-complexed amylose in B-type granules of this genotype. L·AM complexes physically anchor amylose chains together, resisting the ingress of water and creating a network that suppresses swelling even at temperatures that would normally disrupt crystalline order. The high-amylose genotype thus represents a case where granule chemistry overrides the expected size-driven behavior.

6.2 | Amylose Leaching Patterns

At 90°C, AML across all barley starch types follows the order high-amylose > normal > waxy, consistent with total amylose content. In isolated small B-type granules, this same order holds. But in isolated large A-type granules, a different pattern emerges: normal > high-amylose > waxy. The suppression of AML from high-amylose A-type granules despite their high total amylose content points to extensive amylose–amylose and amylose–amylopectin chain interactions within those granules that retain amylose even at elevated temperatures.

Importantly, Myllrinen et al. [9] found that below 90°C, amylose is preferentially released from large A-type granules of normal barley starch, while more amylopectin is released from small B-type granules. This observation reflects the lower crystallinity of A-type granules, allowing easier amylose escape at lower temperatures, while the high L·AM content of B-type granules favors amylopectin release before full gelatinization disrupts their interior organization.

7 | Pasting Characteristics

Pasting behavior how starch viscosity changes during heating, holding, and cooling in water is arguably the most practically important functional property for food and industrial applications. Measured using a Rapid Visco Analyser (RVA) or Brabender Amylograph, pasting analysis yields peak viscosity, breakdown viscosity, setback viscosity, final viscosity, and pasting temperature.

7.1 | Peak Viscosity

Across all three genotypes (normal, waxy, and high-amylose), small B-type granules consistently produce higher peak viscosities than large A-type granules. This finding is surprising; one might expect larger granules to swell more dramatically and create more viscous pastes. The explanation lies in the combination of B-type granules' higher surface area-to-volume ratio (creating a larger effective hydrodynamic volume per unit mass upon swelling) and their higher L·AM content, which produces swollen granules with greater rigidity that resist deformation and therefore contribute more to bulk viscosity. Waxy genotypes show substantially higher peak viscosities than normal or high-amylose types in both granule size classes, consistent with the absence of amylose network-forming constraints on granule swelling.

7.2 | Setback: A Genotype-Dependent Reversal

Setback viscosity, the increase in viscosity upon cooling from the hot paste, reflects the tendency of starch chains to re-associate and partially crystallize. This property shows a striking genotype-dependent reversal between granule sizes. In normal barley starch, setback is higher for large A-type granules than for small B-type granules. In waxy and high-amylose starches, this relationship inverts: small B-type granules show higher setback than large A-type granules.

This reversal has been attributed to differences in amylopectin short-chain distribution, L·AM complex content, and granule crystallinity across the two size classes and three genotypes. Understanding these interactions is directly relevant to designing barley starch-containing products with specific texture, staling resistance, or retrogradation properties.

Table 4. Pasting characteristics of A- and B-type barley starch granules across genotypes.

Granule Type	Peak Viscosity	Setback	Thermal Stability	Shear Stability	Reference
Normal Small (B)	Higher than Large	Lower than Large	Moderate	Moderate	Vasanthan and Bhatty [1]
Normal Large (A)	Lower than Small	Higher than Small	Moderate	Moderate	Vasanthan and Bhatty [1]
Waxy Small (B)	Higher than Large	Higher than Large	Lowest	Lowest	Vasanthan and Bhatty [1]
Waxy Large (A)	Lower than Small	Lower than Small	Lowest	Lowest	Vasanthan and Bhatty [1]
High-amylose Small (B)	Higher than Large	Higher than Large	Highest	Highest	Vasanthan and Bhatty [1]
High-amylose Large (A)	Lower than Small	Lower than Small	Highest	Highest	Vasanthan and Bhatty [1]
Hull-less Waxy A-type	Higher breakdown viscosity	Higher setback	Lower	Lower	Sun et al. [10]

Note: Data synthesized from Vasanthan and Bhatty [1] and Sun et al. [10]. Higher peak viscosity reflects greater water-binding capacity and granule rigidity upon swelling.

8 | Enzyme Susceptibility and Digestibility

8.1 | Alpha-Amylase Hydrolysis Patterns

The susceptibility of barley starch to amylolytic enzymes is not a single, uniform property; it varies markedly between granule size classes, across genotypes, and between different enzyme systems. Three enzyme preparations have been most thoroughly studied: Porcine Pancreatic A-Amylase (PPA), bacterial α-amylase from Bacillus species (BAA), and amyloglucosidase from Aspergillus niger (AAG).

After 72 hours of hydrolysis, PPA preferentially attacks waxy starch (97% hydrolysis) over normal (94%) and high-amylose (91%) types. BAA and AAG show a different pattern: waxy > high-amylose > normal, suggesting that these enzymes interact differently with the amylose matrix than PPA. Across all three enzyme systems and all genotypes, the consistent finding is that small B-type granules are hydrolyzed more rapidly and to a greater extent than large A-type granules under the same conditions.

The mechanistic basis for this higher susceptibility is multifactorial. B-type granules offer more enzyme binding sites per unit mass due to their higher surface area-to-volume ratio. Their surface pores and internal channels facilitate enzyme penetration into the granule interior. And while their lower RC exposes more amorphous regions to enzymatic attack, the higher L·AM content of B-type granules eventually acts as a barrier that slows but does not prevent deeper hydrolysis.

Table 5. Enzyme susceptibility of A- and B-type barley starch granules.

Enzyme / Starch Type	Normal (%)	Waxy (%)	High-amylose (%)	Granule Size Effect	Reference
PPA (72 h) combined	94	97	91	Small > Large	Li et al. [2]
BAA (72 h) combined	29	78	37	Small > Large	Li et al. [2]
AAG (72 h) combined	30	67	33	Small > Large	Li et al. [2]
α-amylase B-type (hull-less)	Higher initial rate	Higher initial rate	—	B-type > A-type (initial)	Sun et al. [10]
A-type hydrolysis (mashing)	Complete at 62°C/10 min	Complete at 62°C/10 min	—	A-type hydrolyzed first	Rittenauer et al. [11]
B-type hydrolysis (mashing)	Partial at 62°C, persists to 78°C	Partial	—	B-type resists hydrolysis	Bühler et al. [12]

Note: PPA= PPA; BAA= Bacillus species α-amylase; AAG= amyloglucosidase from Aspergillus niger. Mashing hydrolysis data from malting industry studies.

8.2 | Industrial Implications: Brewing and Bioethanol

In malting and brewing, the differential gelatinization and hydrolysis of A- and B-type granules have direct practical consequences. A-type granules gelatinize and are hydrolyzed within 10 minutes at standard mashing temperatures (61.4–64.5°C), yielding close to the maximum fermentable sugar output. B-type granules resist complete gelatinization at these temperatures, persisting into the high-temperature mashing range (72–78°C) and yielding relatively more dextrin and non-fermentable carbohydrates that affect beer body and flavor without contributing to fermentation efficiency.

For bioethanol production, the persistence of B-type granules during saccharification has similar implications. Although B-type granules account for only approximately 8% of total starch by mass, their resistance to hydrolysis under standard conditions can disproportionately limit fermentable sugar yield if mashing temperatures or enzyme dosing are not optimized. Supplementary glucoamylase addition or higher mashing temperatures specifically targeting B-granule starch represent practical engineering solutions worth systematic evaluation.

9 | Retrogradation Behavior and Gel Properties

Once gelatinized starch cools and is stored, its chains begin to re-associate through hydrogen bonding, a process called retrogradation. Amylose forms double helices rapidly (within hours), while amylopectin re-crystallizes more slowly through association of its outermost short branches (DP $\leq$ 15). Retrogradation is responsible for bread staling, changes in frozen food texture, and, when deliberately controlled, the formation of resistant starch type 3 (RS3).

9.1 | Retrogradation Enthalpy Across Genotypes

DSC measurements show that retrogradation enthalpy (ΔHR) after 2–4 days of storage at 6°C follows the order high-amylose > waxy > normal. This trend is intuitive for waxy starch (which retrogrades primarily through slower amylopectin re-crystallization) and for high-amylose starch (where abundant free amylose chains provide ample substrate for rapid amylose double-helix formation). The normal starch, with intermediate amylose content, retrogrades to an intermediate extent.

9.2 | Granule Size Effects: a Methodological Contradiction

The effect of granule size on retrogradation is one of the more contentious topics in this field, largely because different measurement approaches yield apparently contradictory conclusions. DSC measurements on normal barley starch show that small B-type granules retrograde more slowly than large A-type granules. This observation is consistent with the higher L·AM content of B-granules impeding chain mobility and amylose–amylose re-association.

However, turbidity measurements, which track light scattering from developing crystallites and aggregates, show the opposite: small B-type granules are the easiest to retrograde in both normal and waxy starches. This apparent contradiction likely reflects the fact that DSC and turbidity measure different aspects of retrogradation. DSC captures large-scale crystalline reorganization requiring extensive chain mobility, while turbidity responds to shorter-range aggregation and early nucleation events. These two processes can proceed at different rates, and the 'easier' nucleation of B-type granule chains may generate turbidity without producing large DSC-detectable crystalline domains.

9.3 | Gel Network Structure

Rheological studies on hull-less barley A- and B-type starch gels show that B-type gels have a lower storage modulus (G') than A-type gels, indicating a weaker, less elastic network. This finding is consistent with B-type granules' slower crystalline retrogradation and has direct implications for food product texture: in applications where gel strength is important, such as puddings, sauces, and gelled confectionery, A-type granule fractions would be expected to produce firmer, more stable textures than B-type fractions.

Table 6. Retrogradation enthalpy and granule size effects in barley starches.

Barley Starch / Genotype	ΔHR (J/g) 2 days	ΔHR (J/g) 4 days	Granule Size Effect	Reference
Normal	10.3	10.6	Large > Small (DSC); Small > Large (turbidity)	Fredriksson et al. [13] Myllarinen et al. [9]
Waxy	10.6	11.1	Small > Large (turbidity)	Fredriksson et al. [13]
High-amylose	12.5	13.1	—	Fredriksson et al. [13]
B-type (hull-less barley)	Lower G'	Slower retrogradation	B-type < A-type (rheology)	Lv et al. [10]

Note: ΔHR expressed as J/g amylopectin. G' = storage modulus. Retrogradation % (R) for malting varieties = ΔHR/ΔHG × 100.

10 | Resistant Starch Formation and Nutritional Significance

10.1 | Classification and Health Relevance

Resistant Starch (RS) is the fraction of dietary starch that escapes enzymatic digestion in the human small intestine and reaches the colon intact, where it undergoes fermentation by the resident microbiota. This fermentation produces short-chain fatty acids (particularly butyrate) associated with improved colonic health, reduced colorectal cancer risk, enhanced satiety, and prebiotic effects that support beneficial bacterial populations. RS is broadly classified as RS1 (physically inaccessible starch), RS2 (native crystalline granules), RS3 (retrograded amylose), and RS4 (chemically modified starch).

10.2 | B-type Granules as RS2 Candidates

The higher RC, greater L·AM complex content, and elevated gelatinization temperatures of B-type granules compared to A-type granules make them plausible preferential contributors to the RS2 fraction in native barley starch. Under typical food cooking temperatures (≤80°C), a measurable fraction of B-type granules may survive without fully gelatinizing, remaining structurally intact when they reach the small intestine and thus contributing to RS2. This hypothesis is physiologically plausible but has not yet been rigorously tested using isolated, size-fractionated granule populations in in vitro or in vivo digestion models.

10.3 | RS3 Enhancement Strategies

In high-amylose barley starch (HABS), RS3 content increases from 3.8% in native starch to 7.0% upon storage at 20°C, and can be further elevated to 13.4–20.2% through annealing (three cycles at 100°C) and/or pullulanase (debranching enzyme) pretreatment. Acid hydrolysis prior to annealing produces RS3 content of 21.5% in HABS comparable to enzymatic debranching but potentially more economically viable at industrial scale. The highest RS values reported for barley starch come from genetically engineered amylose-only varieties (starch branching enzyme RNAi lines), with RS contents of 90% in native starch and 65–68% in gelatinized or retrograded starch values that dwarf anything achievable through processing of conventional genotypes.

Table 7. Resistant starch (RS3) content under different processing conditions.

Starch / Treatment	RS3 Initial (%)	RS3 After Treatment (%)	Key Observations	Reference
HABS native	3.8	—	Lower than amylomaize	Vasanthan and Bhatty [1]
HABS stored at 20°C	3.8	7.0	Significant increase on storage	Vasanthan and Bhatty [1]
HABS annealing at 100°C (3 cycles)	7.0	13.4	Best RS3 yield from heat treatment	Vasanthan and Bhatty [1]
HABS pullulanase + annealing	8.2	20.2	Highest RS3 for conventional barley	Vasanthan and Bhatty [1]
HABS acid hydrolysis + annealing	—	21.5	More economical than pullulanase	Vasanthan and Bhatty [1]
Amylose-only barley (SBE RNAi) native	90	—	Highest RS reported for any barley	Carciofi et al. [14]
Amylose-only barley gelatinized	65	—	Still markedly higher than normal	Carciofi et al. [14]

Note: HABS= high-amylose barley starch; SBE= starch branching enzyme; RS3= retrograded amylose resistant starch.

11 | Industrial Applications and Granule-Size Considerations

Despite barley starch occupying a modest share of the global commercial starch market compared to maize, wheat, and potato, several countries, most notably Finland, maintain active wet-milling operations that naturally produce A-starch (prime starch, predominantly large granules) and B-starch (secondary fraction, predominantly small granules). This industrial separation is an implicit acknowledgment that granule size matters for functional value.

A-type prime starch is used in paper coating and textile sizing, where its high purity and consistent paste properties are valued. B-starch fractions have historically been considered lower-grade, used in animal feed or as fermentation substrates. However, emerging evidence that B-type granules possess higher peak viscosities, greater enzyme resistance under mild conditions, and potentially higher RS2 content suggests that B-fractions may be underutilized and warrant re-evaluation for specialty food and nutraceutical applications.

Table 8. Industrial applications of barley starch and granule-size implications.

Application Sector	Preferred Granule Type	Genotype Preference	Rationale
Brewing / Malting	A-type (primary target)	Normal / waxy	A-granules gelatinize and hydrolyze first; B-granules persist and yield dextrins affecting beer fermentability.
Bioethanol production	Both (optimize B-granule hydrolysis)	Waxy / Normal	B-granule resistance reduces fermentable sugar yield; temperature optimization or enzyme supplementation needed.
Paper / Textile sizing	A-type (prime starch)	All genotypes	Large A-granules yield high-purity prime starch for coating and sizing (Finnish commercial operations)
Food thickening/gelling	A-type preferred	Waxy / Normal	A-granules contribute to stronger gels; waxy genotype provides clear, cohesive pastes.
Resistant starch / Functional food	B-type (higher SA/V ratio)	High-amylose	Higher crystallinity, L·AM content, and gelatinization temperature of B-granules favor RS2 formation.
Animal feed / Nutrition	Both	All genotypes	Waxy starch digests most rapidly; high-amylose types provide slower energy release.
Cationic starch (paper industry)	Both	Normal / Waxy	DS comparable to maize; waxy barley cationic starch shows superior paper strength properties.

Note: DS= degree of substitution. Applications reflect commercial practices of Finnish barley starch producers and academic literature synthesis.

12 | Chemical and Physical Modification

12.1 | Chemical Modification

Native starches are often inadequate for industrial applications because they are sensitive to heat, shear, acid conditions, and freeze-thaw cycling. Chemical modification introducing functional groups onto starch chains addresses these limitations. For barley starch specifically, cationization, crosslinking, and hydroxypropylation have received the most attention, though nearly all published work uses bulk (non-fractionated) starch rather than isolated A- or B-type granule fractions.

Cationization with 3-chloro-2-hydroxypropyl-trimethylammonium chloride in alkaline alcoholic media produces similar degrees of substitution (DS = 0.039–0.044) across normal, waxy, and high-amylose barley starches comparable to normal maize (DS = 0.044). Cationic barley starch shows superior paper strength properties over commercial cationic maize starch, indicating commercial potential. Crosslinking of waxy barley starch with phosphorus oxychloride requires less reagent than equivalent maize starch modification (0.005–0.007% vs. 0.010–0.015%), and subsequent hydroxypropylation (MS 0.10–0.13) imparts freeze-thaw stability surpassing commercially modified waxy maize starch. The question of whether isolated A- or B-type granules respond differently to these modification chemistries in terms of DS achieved, granule morphological changes, and functional outcome remains a significant open question.

12.2 | Annealing

Annealing, holding starch below its gelatinization temperature at high moisture (typically 50°C for 72 hours at 75% moisture content), permits molecular reorganization without granule disruption. For barley starch, annealing generally increases RC, narrows the gelatinization temperature range, raises transition temperatures, and reduces swelling. High-amylose genotypes respond distinctively, showing increased gelatinization enthalpy after annealing (other genotypes show no enthalpy change), and converting from an A-type to mixed A+B X-ray diffraction pattern in both normal and high-amylose genotypes. How isolated A- and B-type granule fractions respond individually to annealing remains unreported.

13 | Research Gaps and Future Directions

As this review makes clear, the body of knowledge on A- and B-type barley starch granule properties is substantial, but it is also noticeably incomplete in several areas that matter for industrial and nutritional applications. We highlight four priority knowledge gaps.

Chemical modification of size-fractionated granules: No published study has systematically characterized how isolated A- and B-type granules respond to crosslinking, cationization, acetylation, or hydroxypropylation. Given that B-type granules have higher surface area, greater lipid content, and different chain length distributions, their reactivity toward chemical reagents and the functional properties of the resulting modified starches almost certainly differ from A-type granules. This gap limits precision in designing modified barley starches for specific applications.

Physical modification (annealing) of isolated granule fractions: The annealing behavior of isolated A- and B-type granules from different genotypes has not been reported. Given their different crystalline architectures and L·AM contents, each granule population is likely to respond differently to annealing temperature, duration, and moisture with implications for how annealing can be used to tune functional properties in size-fractionated applications. Freeze-thaw stability of size-fractionated granules: Available freeze-thaw data are limited to bulk starches. A systematic study of isolated A- and B-type granule fractions from all three genotypes, before and after chemical modification, would substantially improve the evidence base for designing barley starch-containing frozen food products.

Multi-scale structure–function integration: Recent advances in SAXS, Small-Angle Neutron Scattering (SANS), and Atomic Force Microscopy (AFM) offer unprecedented resolution of lamellar thickness, double helix orientation, and surface topology in individual granules. Integrating these structural measurements with functional testing pasting, gelation, digestibility in size-fractionated granule populations would bridge a longstanding gap between nanostructural observation and macroscopic functional behavior.

14 | Conclusion

The bimodal granule size distribution of barley starch is not merely a morphological curiosity; it is a functionally consequential feature that makes barley starch one of the most structurally interesting cereal starches available to food scientists and industrial processors. As this review has demonstrated, A- and B-type granules are not simply differently sized versions of the same particle. They are compositionally, structurally, and functionally distinct entities whose behavior is further shaped by whether they come from normal, waxy, or high-amylose genetic backgrounds.

B-type granules consistently show higher gelatinization temperatures, lower gelatinization enthalpies, greater initial enzyme hydrolysis rates, and higher peak viscosities than their A-type counterparts, all of which can be traced, to a considerable degree, to their higher content of lipid-complexed amylose and their greater surface area-to-volume ratio. Yet these same properties do not simply scale uniformly across genotypes: swelling behavior, setback viscosity, and AML all exhibit genotype-dependent reversals that add layers of complexity to any attempt at simple generalization.

From an industrial and nutritional standpoint, the implications of this heterogeneity are significant. In brewing, the persistence of B-type granules beyond standard mashing temperatures contributes to dextrin formation and affects beer fermentability a challenge that could be addressed through mashing temperature optimization or supplementary enzyme use. In functional food development, the higher crystallinity and thermal stability of B-type granules make them attractive candidates for RS2-enriched products, though this potential has yet to be systematically tested. And across food applications where freeze-thaw stability or gel strength are important, the differential retrogradation and gelation behaviors of the two granule populations offer opportunities for targeted formulation if fractionated starch can be produced cost-effectively.

Realizing the full potential of barley starch's granule size heterogeneity will require systematic research on size-fractionated materials, particularly in areas of chemical modification, physical modification, and freeze-thaw stability where the published evidence remains sparse. With these gaps addressed, the prospect of deploying barley starch in highly targeted food, nutraceutical, and industrial applications becomes substantially more achievable.

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Conflict of interest

The authors declare no conflict of interest.

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