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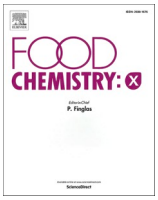

Evaluation of amylose content: Structural and functional properties, analytical techniques, and future prospects

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ABSTRACT

Amylose content (AC) is critical in evaluating starch properties, significantly influencing the food industry and human nutrition. Although amylose is not completely linear, its unique structure makes it a key research focus across various scientific fields. Understanding amylose's structural and functional properties is essential for its applications in medical, nutritional, and industrial sectors. Accurate determination of AC, from simple qualitative assessments to precise quantitative measurements, is vital for effectively processing and using starch-rich products. The choice of AC determination method depends on the specific application and the required accuracy and detail. This review summarizes amylose's structural and functional characteristics and recent advancements in qualitative and quantitative AC determination techniques. It also provides insights into future trends and prospects for these technologies, emphasizing the need for more rapid, convenient, accurate, and customizable methods. In conclusion, advancements in amylose determination should enhance accuracy, speed, and ease of use to improve quality control and applications across various sectors while expanding our understanding of amylose and its functionalities.

1. Introduction

Starch comprises two constituents: amylose, which has slight branching, and amylopectin, which is highly branched (Wang, Vilaplana, Wu, Hasjim, & Gilbert, 2019). Amylopectin consists of α-D-glucose molecules linked by α-(1/4) and α-(1/6) glycosidic bonds, with branching occurring approximately every 6 to 100 monomers (Khatami, Barber, & de Haan, 2021). In contrast, amylose comprises glucosyl units linked to α-D-glucose units linked by α-(1/4) glycosidic bonds. The length of these chains typically ranges from about 600 to as many as about 18,000 glucose units (Khatami et al., 2021). The internal organization of starch is characterized by a hierarchical radial structure consisting of alternating concentric rings of amylose and amylopectin. Within these rings, amylopectin forms two-fold helices, known as crystalline lamellae, while amylose forms tightly packed single helices, forming amorphous lamellae (Fig. 1) (Wang, Xu, & Luan, 2020), and efforts have been undertaken to elucidate the branching structure of amylose. Nevertheless, the precise nature of amylose branching, which varies depending on the species and variety, remains incompletely understood (Wang et al., 2019).

The amylose-to-amylopectin ratio is a pivotal factor affecting the physicochemical properties of starch (Xu et al., 2024). This ratio influences the digestibility, dispersibility, and rheological properties of starch, which are crucial for its application in food and industrial products. High amylose content (AC) generally correlates with increased resistance to digestion and enhanced mechanical properties, making it a valuable component in developing new starch-based materials (Varghese et al., 2023). Also, the degree of branching and the presence

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of long amylose chains play a critical role in determining the structural order and thermal stability of starch granules (Tian et al., 2024). High amylose starches with abundant branched internal long chains (B2 + B3) allow for easy entanglement with other molecular chains, resulting in a more compact structure and higher resistance to enzymatic hydrolysis (Gu et al., 2024). Furthermore, amylose inclusion complexes (AICs) have gained significant interest in culinary applications in recent years. Due to their resistance to enzymatic breakdown, they act as dietary fibers (Di Marco, Ixtaina, & Tomás, 2022). Additionally, their potential as delivery systems has been highlighted, given their ability to encapsulate molecules that are valuable for food applications (Zhang et al., 2021).

Recently, there has been a tendency to find more modern and accurate techniques to accurately estimate and determine the AC in plant sources to keep pace with the requirements of scientists and researchers to reach useful results for developing various industries that depend on amylose. Qualitative methods are a simple indicator of amylose's presence or absence in a starch sample, making it possible to visually assess the presence of amylose based on color change (Kim & Jung, 2022). On the other hand, semi-quantitative approaches may give a reasonable estimate of the relative quantity of amylose in a sample. These approaches are less accurate than quantitative approaches but more sensitive than qualitative ones, such as spectrophotometric techniques (Bahdanovich, Axelrod, Khlystov, & Samburova, 2022). In contrast, the exact quantity or proportion of amylose in starch samples may be determined using quantitative techniques. These techniques are appropriate for in-depth analysis and study because of their high repeatability, accuracy, and sensitivity levels. Gravimetric procedures, enzymatic tests, and high-performance liquid chromatography (HPLC) are common quantitative approaches (Subroto, Jeanette, Meiyanasari, Luwinsky, & Baraddiaz, 2020). However, many techniques used for AC determination have been reported and have generated different performances in various starches or starch-rich materials regarding sensitivity, accuracy, and availability.

However, the primary objective of this review is to offer a thorough and detailed evaluation of amylose, focusing on its structural and functional properties and its relevance in the food industry, human nutrition, and various scientific fields. Moreover, this review aims to elucidate the various methods employed for determining AC, critically analyzing each method's benefits, limitations, and practical applications. By categorizing these methods into qualitative, semi-quantitative, and quantitative approaches, the review aspires to serve as a comprehensive resource, guiding researchers and professionals in making informed decisions based on the most suitable analytical techniques.

2. Distinct characteristics of amylose

Amylose possesses unique properties that make it a versatile and valuable component across various applications. Its diverse structural and functional characteristics, biocompatibility, and potential for targeted drug delivery underscore its significance in the pharmaceutical and food industries, opening up promising opportunities for innovative applications and enhanced therapeutic results. This section provides a concise overview of the structural, physicochemical, and functional properties of amylose. Table 1 highlights the various impacts of amylose on the functional and physicochemical properties of starch.

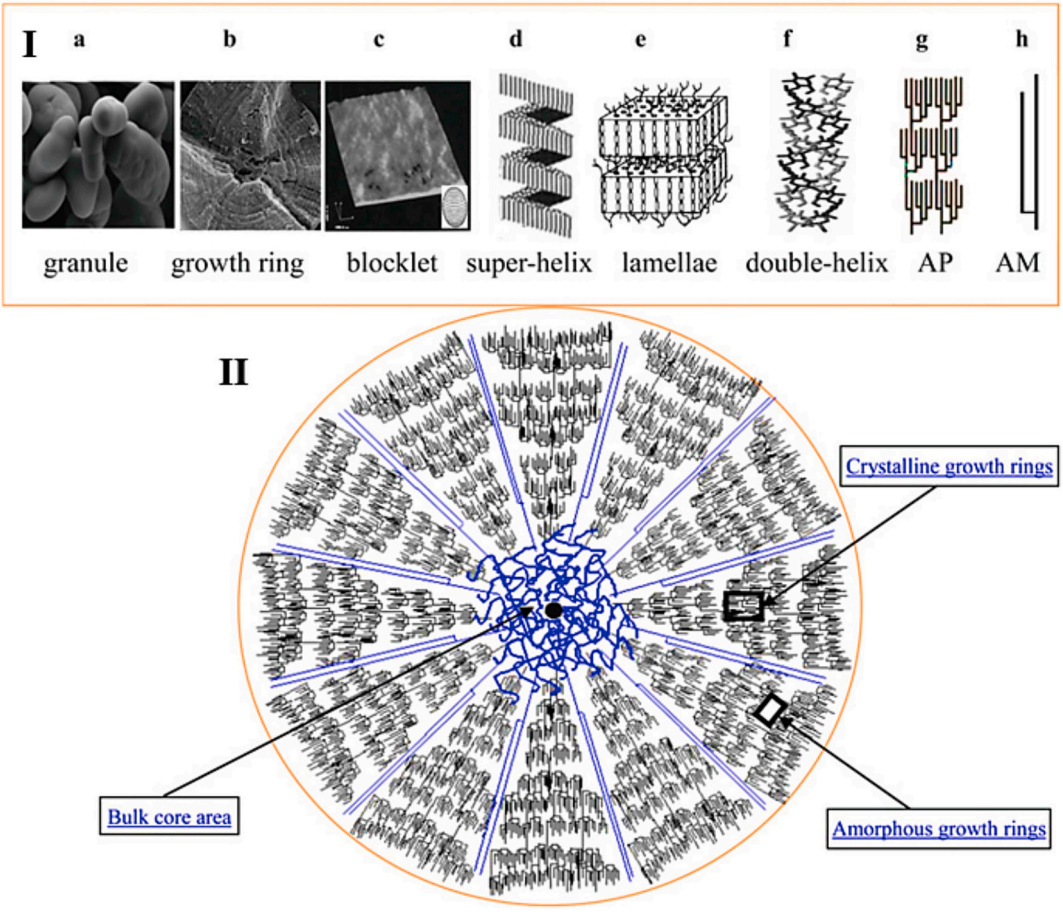

Fig. 1. Starch granules and their distinct structures (Adapted from Wang et al., 2020, reproduced by permission from Elsevier).

Table 1
Varied impacts of amylose on the functional, physical, and chemical characteristics of starch.

Sources	Main contribution	Affected properties	Reference
Sago starch	The process of fractionating sago starch is carried out using hot water and the addition of n-butanol dropwise with certain time variations (1, 2, 3, 4, and 5 h).	• The amylose in sago starch affects gelatinization, retrogradation, and pasting. • Fractionation with n-butanol increases AC, particle size, and thermal stability while decreasing crystallinity, water solubility, and swelling power in fractionated starches. • Adding n-butanol for 4 h achieves the highest AC and gelatinization temperature.	(Ningrum et al., 2023)
Cross-linked rice starches	The influence of chemical modification using POCl3 on the rice starch function was evaluated.	• The AC influences water solubility, absorption, pasting viscosity, and freeze-thaw stability of cross-linked rice starches, with high-amylose starch showing distinct properties compared to low-amylose starches. • Cross-linking increased pasting viscosity and decreased paste clarity. • High-amylose starch showed better stability and viscosity control. • Cross-linking with POCl3 affects rice starch physicochemical properties.	(Bagheri, Radi, & Amiri, 2018)
High-amylose starches	Summarize high-amylose starch's development, structure, and nutritional functionality and identify molecular features contributing to digestive enzyme resistance in high-amylose starch.	• High-amylose starchs exhibit unique functional properties like resistance to digestive enzymes, forming dense structures during heat treatment, and the potential for improved nutritional outcomes in food products. • High-amylose starchs resist digestive enzyme degradation during heat treatment. • High-amylose starchs have unique functional properties and enhanced nutritional values.	(Li, Gidley, et al., 2019)
Single amylose chains	The structure of amylose chains in water using molecular dynamics simulations was studied to identify and characterize these “imperfect” helical structures, and geometry-based criteria were devised to define imperfect helices based on length and temperature.	• Amylose exhibits dynamic helical structures with imperfect H-bond patterns in water, influenced by chain length and temperature, showcasing flexibility and transient helicity distinct from polypeptides. • Helices in amylose chains are dynamic and short-lived • The analysis uncovers short and long helix-breaking mechanisms such as band-flips and kinks in the chain. • Helical conformations are a key secondary structure for amylose chains in water.	(Khatami et al., 2021)
High-amylose starch	Study the various forms and processing techniques used to produce high-amylose starch based polymers and composites addressing their favorable properties compared to normal starch.	• Amylose exhibits altered solubility reduced swelling, and can be modified chemically or physically to enhance specific properties, making it valuable for structural applications in biodegradable materials. • High-amylose starch-based polymers have unique properties and enhanced nutritional values. • Blending high-amylose starch with other biomaterials can improve its properties.	(Faisal, Kou, Zhong, & Blennow, 2022)
Black rice	The color, crystalline structure, and swelling properties of parboiled rice with different AC were analyzed, and the water molecular mobility, texture, and starch digestibility of cooked rice were determined.	• The physicochemical properties of parboiled black rice varied with AC. • Waxy and medium-amylose rice had damaged crystalline structures. • High-amylose rice retained its crystalline structure after parboiling. • Cooked high-amylose rice had higher resistant starch content.	(Zhang et al., 2022)
Grafted spiropyran-amylose (ASP)	The photochromic spiropyran-amylose (ASP) biobased polymers with different grafting densities were synthesized through azide-alkyne cycloaddition of an alkynyl-functional SP derivative onto C6-azidated amylose, where the unconverted azide in ASP40PA60, ASP100, and the AN3 precursor are quenched by clicking with propargyl alcohol (PA).	• The grafted spiropyran-amylose (ASP) biobased polymers exhibit photochromism, solubility in specific solvents, reversible switching between SP and MC states, and affected wettability in coatings. • Grafted spiropyran affects the solubility and film properties of amylose. • Grafted SP switches to zwitterionic MC, affecting wettability minimally. • Photochromic spiropyran-amylose polymers with different solubility and film properties. • Poor solubility in trifluoroethanol, ethanol, and water. • Limited solubility in low-polarity tetrahydrofuran.	(Barsi et al., 2023)
High-amylose rice	The postprandial glycemic response and physical features of high-amylose rice and the effect of cooking conditions on blood glucose levels at various intervals after each meal were assessed.	• High-amylose rice exhibits favorable postprandial glycemic responses and physical properties, such as reduced blood glucose variation and improved sensory evaluation, making it beneficial for health and cooking applications. • Adding 5 % trehalose to High-amylose rice improved physical properties and sensory evaluation.	(Yamaguchi, ENOkI, Sasagawa, & Fujimura, 2019)
Amylose synthesized (AS) by amylosucrase	Compare the gene and enzyme characteristics of microbial AS and focus on the applications of AS in the biotechnology and food industries.	• AS by amylosucrase exhibits versatile properties, including forming microparticles for drug delivery, chromatography, and bioanalytical applications, increased water solubility, stability, and production of resistant starches. • AS synthesizes starches, nanoparticles, and bio-functional compounds efficiently.	(Seo, Yoo, Choi, Kim, & Park, 2020)

2.1. Structural properties

2.1.1. Structural composition

The distinctive structure of amylose provides many physicochemical features utilized in food technology (Obadi, Qi, & Xu, 2023), medicines (Chen & Liao, 2022), and materials research. Structurally, amylose is typically the second most abundant component of starch, accounting for 20–30 % of its weight, and is generally recognized as a linear or slightly branched molecule with a degree of polymerization (DP) ranging from approximately 1000 to 5000, depending on the source of the starch (Agnihotri, Jain, Vr, Jadhav, Gomte, & Jain, 2024). Molecular characteristics of amylose, such as its molar mass and purity, have revealed a narrow range of molar mass distribution and high purity levels ranging from 85.6 to 92.6 % (Reyniers et al., 2020). On the other hand, experimental research has shown that amylose chains form helical structures in both single- and double-stranded polymorphs. Double-stranded amylose chains can crystallize into two primary forms: A and B (Villwock & BeMiller, 2022). The amylose chains produce left-handed helices in both crystal forms, resulting in intermolecular hydrogen bonds between the two strands (Fig. 2) (Mohamed, 2021). In contrast, single-stranded amylose, often called V-amylose, does not form helical structures in pure water. Instead, it forms these structures in the presence of iodine, dimethyl sulfoxide (DMSO), alcohols, or fatty acids (Khatami et al., 2021). The V-type helical shape of amylose and its complexes with guest molecules prevent water absorption into the amylose cavity, thereby increasing stability (Wang et al., 2023). Also, the composition of amylose varies across plant species, affecting its molecular weight distribution and branching patterns, which in turn influence the functional characteristics of starch (Seung, 2020). Molecular dynamics simulations indicate that, despite suboptimal hydrogen bonding, amylose chains can adopt helix-like shapes in water. These properties enable amylose to form stable structures and disperse in water, making it versatile for various applications.

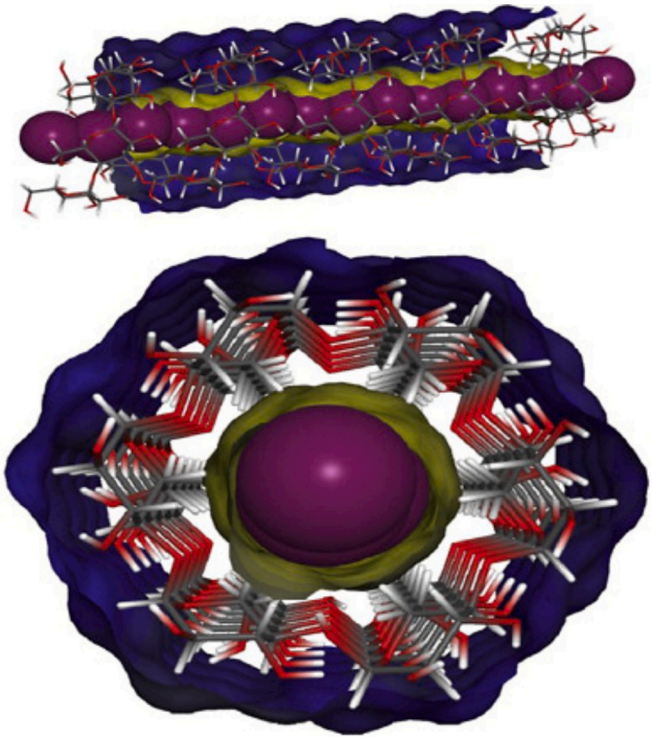

Fig. 2. Left-handed single helix of V-amylose, showcasing a distinctive central hydrophobic channel containing polyiodide (highlighted in purple). Atom representation employs CPK coloring, with blue and green indicating hydrophilic and hydrophobic surfaces. (Adapted from Mohamed, 2021, reproduced by permission from Elsevier). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.1.2. Theories of amylose branching

The study of amylose branching encompasses various theories and hypotheses that aim to understand and manipulate the branching process. These theories delve into enzymatic actions, synthetic methodologies, and structural analyses, providing insights into how amylose can be branched to form amylopectin-like structures or other branched polysaccharides (Li, Yu, Dhital, Gidley, & Gilbert, 2019). One of the key enzymatic mechanisms of amylose branching is known as the Potato Q-Enzyme Mechanism. This mechanism involves the potato Q-enzyme facilitating the conversion of amylose into an amylopectin-like molecule through a random, endo-type transglycosylation process. During this process, the enzyme catalyzes the transfer of a portion of one amylose chain to another, effectively creating branches. The substrate must have a stabilized secondary and tertiary structure for the enzyme to function, which is crucial for inter-chain transfer (Miao & BeMiller, 2023). The mechanism suggests that the enzyme may form an intermediate with a donor chain, which then reacts with an acceptor chain. Alternatively, it may interact with a complex of two chains, potentially forming a double helix structure supporting the branching process. Another significant enzymatic mechanism is the cyclization by branching enzymes (Guo et al., 2021). In this process, the branching enzyme produced by some bacteria, such as *Bacillus stearothermophilus, Geobacillus stearothermophilus,* and *Bacillus caldolyticus*, which often contains a carbohydrate-binding module 48, catalyzes the cyclization of amylose, resulting in a cyclic glucan with both alpha-1,4- and alpha-1,6-glucosidic linkages (Ban et al., 2020). This cyclization reduces the molecular size of amylose without increasing its reducing power, indicating a deviation from the conventional model of amylose branching. The cyclic nature of the product is confirmed through mass spectrometry and its resistance to glucoamylase digestion, which underscores the uniqueness of this enzymatic reaction. Beyond enzymatic mechanisms, synthetic methodologies have been developed to achieve controlled branching in amylose. One notable approach is regioselective branching in amylose esters. This method involves a novel one-pot synthesis, allowing precise control over regioselective branching (Thompson & Edgar, 2024). The process includes bromination at the C6 position of amylose, esterification at other hydroxy groups, followed by azide substitution, and a click reaction to produce branched polysaccharide derivatives. This technique enables the creation of well-defined, comb-like structures with controlled branching, offering significant potential for tailored applications in various industries. To assess the branching ratios of starch, which includes both amylose and amylopectin content, proton nuclear magnetic resonance (NMR) is commonly used. This technique identifies and quantifies branching in starch molecules (Van Leeuwen et al., 2021). This analysis provides a framework for understanding the structural diversity of amylopectin and its implications for starch properties, particularly in relation to its functionality in food applications. While these studies offer significant insights into the mechanisms and methodologies of amylose branching, challenges remain in fully understanding the implications of branching on the functional properties of starches. The enzymatic and synthetic approaches discussed highlight the complexity of polysaccharide chemistry and the potential for innovation in this field. However, there is still much to explore, particularly in developing more efficient and versatile methods for controlling amylose branching. Future research should focus on these aspects to enhance our understanding of how amylose structure influences its functional properties and applications across various industries, particularly in food science.

2.2. Physicochemical properties

2.2.1. Thermal properties

AC affects the thermal behavior of starch-based materials, with high amylose ratios leading to enhanced thermal insulation performance

(Han et al., 2023). Research has shown that high-amylose starches are characterized by their unique thermal properties, which are influenced by their molecular structure. The degree of molecular branching and the extent of V-type crystalline polymorphs are critical in determining these properties. For instance, high-amylose starchs from different botanical sources exhibit varying gelatinization enthalpies and temperatures, which depend not solely on AC but also on the structural order within the starch granules (Tian et al., 2024). Furthermore, ohmic heating is a novel processing technique that can enhance the thermal properties of high-amylose starch by increasing granular swelling and reducing resistant starch content (Castro-Campos et al., 2023). Also, the interaction between amylose and other molecules, such as dicarboxylic acids, can form ICs that alter the thermal stability of starch. These complexes exhibit high dissociation temperatures, which can be utilized to modify starch functionality in food applications, enhancing thermal stability and potentially improving food quality (Kenar, Compton, Peterson, & Felker, 2022). In food packaging, starch-based nanocomposites with varying amylose/amylopectin ratios can be engineered to enhance thermal stability. The interaction between amylose and nanofillers affects these materials' structural integrity and thermal properties, making them suitable for sustainable packaging solutions (Zhang, Zhu, Liu, Zou, & Li, 2019). The encapsulation of bioactive compounds in AICs can also significantly enhance their thermal and photostability (Di Marco et al., 2022; Liu et al., 2023). So, the thermal properties of amylose, such as applying hydrothermal treatments to high amylose flour, can be exploited to develop food products with increased dietary fiber content, which is advantageous for creating health-oriented food products.

2.2.2. Polymerization properties

Amylose exhibits unique polymerization properties that are pivotal in forming various supramolecular structures and functional materials. These properties are primarily due to its helical conformation, which allows for forming ICs and double helices. The polymerization characteristics of amylose are influenced by factors such as its DP, the presence of guest molecules, and the method of polymerization, such as enzymatic processes. The DP of amylose significantly affects its structural and functional properties. Higher DP leads to increased crystallinity, molecular density, and thermal stability, influencing the digestion kinetics of amylose-based complexes. These complexes exhibit V-type crystalline diffraction and rod-like molecular configurations, which are crucial for their digestion mechanism and kinetics (Li et al., 2023). Furthermore, the structural variations in amylose, such as its nanoscale polymerization index, play a critical role in forming starch-based multicomponent foods, affecting their nutritional and energy profiles. Also, amylose can form gels through non-covalent cross-linking, facilitated by enzymatic polymerization. This process involves the formation of double helices and ICs, which contribute to the unique properties of the resulting gels (Tanaka, 2018). The enzymatic polymerization of amylose, particularly through phosphorylase catalysis, allows for precisely synthesizing well-defined polysaccharides, forming complex supramolecular networks (Kadokawa, Shoji, & Yamamoto, 2019). On the other hand, amylose forms supramolecular ICs with various guest polymers through a process known as vine-twining polymerization. This method involves the helical inclusion of hydrophobic guest molecules, forming nanostructured materials such as hydrogels and films (Kadokawa, 2023). The vine-twining polymerization process is characterized by the selective inclusion of guest polymers, which can be tailored to form specific supramolecular structures depending on the guest polymer's properties.

2.2.3. Gelatinization and retrogradation properties

Amylose plays a crucial role in starch's gelatinization and retrogradation processes. Gelatinization involves heating starch in water, disrupting its crystalline structure, and allowing amylose and amylopectin to leach out and form a viscous paste. Upon cooling, amylose realigns, leading to retrogradation, which increases the firmness and opacity of starch gels (Karp et al., 2021). This feature significantly influences the texture of various food products like bread and noodles. Retrogradation also contributes to the formation of resistant starch, as amylose plays a pivotal role in the retrogradation process of starch, which is a critical factor affecting the texture and nutritional value of starchy foods (Li & Hu, 2021). Retrogradation involves the reassociation or recrystallization of gelatinized starch components, primarily amylose and amylopectin. The structural characteristics of amylose, such as chain length and molecular weight, significantly influence its behavior during retrogradation, as amylose contributes to the formation of ordered structures. For example, the chain-length distribution affects the degree of interchain entanglement and overlap, which are crucial for the texture of retrograded starch. Shorter amylose chains tend to form more rigid gels due to increased inter-chain entanglement, while longer chains promote more extensive crystalline structures (Li et al., 2022; Zhang et al., 2024). So, the interactions are influenced by the chain-length distribution of amylose, which can either enhance or inhibit the formation of these structures. Amylose-lipid complexes (A-LC) can inhibit retrogradation and modify starch digestibility. These complexes are used in controlled-release drug formulations and functional foods (Di Marco et al., 2022). However, various external factors, such as temperature, moisture, and the presence of other food ingredients, can influence amylose retrogradation. For instance, the addition of konjac glucomannan has been shown to inhibit the retrogradation of amylose by affecting its water-holding capacity and crystallinity (Wang et al., 2022). This suggests that the presence of hydrophilic colloids can modulate the retrogradation process by altering the physical properties of amylose gels (Chiravi, Khan, Wadikar, Mahesh, & Semwal, 2023). Also, the molecular weight of amylose affects its retrogradation behavior. Lower molecular weight amylose tends to have reduced inter-chain overlap, leading to less viscous solutions but more rigid gels upon retrogradation. This is because smaller amylose molecules can permeate more easily into other molecules, enhancing inter-chain entanglement (Li, Ji, Zhang, et al., 2022).

2.2.4. Viscosity properties

Viscosity is a key parameter that reflects the molecular size and conformation of amylose in solution. Amylose exhibits unique viscometric behaviors, which vary significantly among different sources, that are crucial for its applications in food and industrial processes (Ahuja, Lee, Latshaw, & Foster, 2020). The viscosity-enhancing property of amylose is related to its molecular weight and the formation of entangled networks in aqueous solutions. The viscosity properties of amylose are influenced by various molecular mechanisms, including its interaction with proteins, the formation of specific molecular structures, and the presence of other chemical agents, as well as by concentration and temperature (Liu et al., 2021). For example, amylose interacts with proteins such as glutenin and gliadin, which can significantly alter viscosity. Chen, Zuo, Li, Men, and Lian (2024) noted that when wheat amylose is mixed with non-isolated glutenin, hydrogen bonds form between the α-amine of Phe/Leu in glutenin and the hydroxy of C1 in amylose. This interaction leads to a conformational change in amylose from a double helix to a single helix, reducing the viscosity of the mixture. Similarly, maize amylose interacts with α, β, and γ-gliadin fractions, forming hydrogen bonds that disrupt disulfide bonds in gliadin's intermolecular β-sheet structures. This results in a reduced viscosity of the mixture, as the new hydrogen bonds facilitate the hydration of the complex (Ma, He, Zhang, & Lian, 2024). Additionally, maize amylose can significantly reduce the viscosity of protein fractions such as α, β, and γ-gliadin by forming hydrogen bonds and altering the protein network structure. This interaction leads to the breakdown of disulfide bonds in the protein's β-sheet structures, facilitating a reduction in viscosity. The formation of new hydrogen bonds between amylose and gliadin fractions is crucial in this process, as observed in the changes in diffraction peaks during the interaction (Ma et al., 2024). Thermal properties of amylose also play a crucial role in the viscosity of amylose,

as the intrinsic viscosity of amylose has been positively correlated with thermal enthalpy, indicating a relationship between viscosity and thermal properties. In contrast, retrogradation enthalpy values were negatively related to intrinsic viscosity (Zhao, Hofvander, Andersson, & Andersson, 2023). Castro-Campos et al. (2023) claimed that the ohmic heating results in partial gelatinization and a shift to B-type polymorphism, which can improve the textural properties of food products by increasing viscosity and altering the starch's crystalline structure. While the molecular mechanisms discussed provide a simple understanding of amylose's viscosity properties, it is important to consider the broader implications of these findings. For instance, manipulating amylose's viscosity through chemical modifications or environmental conditions can have significant applications in food technology and material science. However, the complexity of these interactions also suggests that further research is needed to fully exploit these properties in practical applications.

2.2.5. Inclusion complexes (ICs)

Amylose can form ICs with various molecules, such as lipids and small organic compounds. These complexes occur when the amylose helices encase guest molecules within their helical structure. Amylose's ability to form ICs with hydrophobic molecules enhances its functionality as a delivery system for bioactive compounds (Esfahani, Häusler, & Mäder, 2022), as these complexes protect sensitive molecules during processing and storage, ensuring their sustained release in the gastrointestinal tract. This property is particularly useful in developing functional foods with improved nutritional value (Di Marco et al., 2022). Additionally, these complexes protect the fatty acids from oxidation, enhancing their shelf life and functional efficacy in food products. For instance, complexes formed under mild conditions with high amylose corn starch showed improved oxidative stability, retaining over 90 % of omega-3 and omega-6 content after accelerated storage tests (Di Marco, Tomás, & Ixtaina, 2024). Furthermore, the encapsulation of vitamin D in AICs significantly increases its photostability and thermal stability, protecting it from degradation due to light and oxygen exposure. This encapsulation also facilitates a controlled release in the gastrointestinal tract, enhancing the bioavailability of vitamin D in functional foods (Liu et al., 2023). In addition, the capacity of amylose to create compounds with amphiphilic or hydrophobic ligands, leading to the formation of V-amylose structures, has important values for nanoencapsulation, alteration of rheological properties, and mitigation of starch retrogradation (Sang, Xu, Zhu, & Narsimhan, 2021).

On the other hand, AICs can act as effective emulsifiers, improving the texture and stability of emulsions in food products. These complexes, particularly those formed with fatty amine salts, exhibit superior emulsifying activity compared to traditional starch-based emulsifiers. They maintain stable emulsions over long-term storage by preventing oil droplet coalescence, which is crucial for maintaining the desired texture in food products (Selling et al., 2024; Selling, Hojilla-Evangelista, & Hay, 2022). Also, forming AICs with long-chain dicarboxylic acids results in structures that can modify starch properties, influencing food products' overall texture and quality. These complexes exhibit high thermal stability, which can be advantageous in food processing applications that require heat treatment (Kenar et al., 2022).

The scalability of AIC formation using industrial techniques such as steam jet cooking and microwave methods suggests potential for widespread application in the food industry. This scalability is crucial for commercially producing functional foods that leverage the benefits of AICs (Di Marco et al., 2022; Selling et al., 2022). While AICs offer numerous benefits regarding stability and texture modification, it is important to consider the potential challenges associated with their use. The effectiveness of these complexes can vary depending on the specific hydrophobic molecule involved and the conditions under which the complexes are formed. Additionally, the sensory attributes of food products, such as taste and mouthfeel, may be affected by incorporating these complexes, necessitating careful formulation and testing to ensure consumer acceptance. Nonetheless, the promising results from current research indicate a strong potential for AICs to enhance the quality and functionality of food products.

2.3. Functional properties

2.3.1. Structure-function relationship in amylose

The structural variability of amylose significantly impacts its functionality in various food applications, influencing properties such as digestibility, thermal stability, and interaction with other food components. This variability arises from differences in AC, chain length, and the ability to form complexes with other molecules. For example, AC influences the crystalline structure and digestibility of starches. High-amylose starches, such as those from Japonica rice, exhibit a C-type crystalline structure, which differs from the A-type found in waxy and normal starches. This structural difference results in higher resistant starch content and lower digestibility, making high-amylose starches suitable for applications requiring slow energy release or dietary fiber enhancement (Luo et al., 2021). Also, amylose's molecular weight and chain length affect its functional properties in food applications. For instance, potato starches with longer amylose branches show different digestibility profiles compared to other sources (Wang, Hasjim, Wu, Henry, & Gilbert, 2014). The fine structure of amylose also affects its behavior during food processing, such as deep-frying. Short amylose chains in potato flakes increase crystallization and water binding, reducing oil uptake and caloric density in potato crisps. This insight can guide the selection of potato cultivars and processing conditions to produce reduced-calorie crisps (Reyniers et al., 2020). On the other side, the helical shape of amylose enables the creation of compounds with lipids and other small molecules, which can alter the functional properties of starch, such as thermal and digestive characteristics (Feng, Junejo, Zhang, Fu, & Huang, 2024). Research has shown that A-LC can reduce the glycemic response of starchy foods, benefiting blood sugar management in diabetic patients (Huang, Chen, Wang, & Zhu, 2020). Additionally, these complexes improve the stability and shelf life of food products by reducing starch's tendency to recrystallize and grow stale.

On the other hand, high-amylose starches are increasingly used to bridge the “fiber gap” due to their resistance to enzymatic digestion. They form dense molecular structures that resist breakdown, contributing to their role as dietary fibers. This property is beneficial for developing food products to improve gut health and manage blood sugar levels (Li, Gidley, & Dhital, 2019). Moreover, high-amylose starches create thicker pastes, which are desirable in specific food applications like sauces and gravies (Bao & Bergman, 2024). Additionally, the conformational flexibility of amylose, as studied through molecular dynamics simulations, reveals its potential to undergo helix-coil transitions. This flexibility may influence its interaction with other food components and its functional properties in food matrices (Koneru, Zhu, & Mondal, 2019). While the structural variability of amylose offers numerous functional benefits in food applications, it also challenges consistency and predictability. The ability to tailor amylose properties through genetic, processing, and formulation strategies can enhance its utility in diverse food products, but it requires careful consideration of the specific application and desired outcomes.

2.3.2. Modification-function relationship in amylose

Cold plasma treatments, such as dielectric barrier discharge and radio-frequency plasma treatments, have emerged as a promising method for modifying the structural properties of amylose, enhancing its thermal stability and resistance to gelatinization, and offering potential enhancements in their functional and structural characteristics (Sifuentes-Nieves et al., 2021). These treatments involve the use of partially ionized gases to induce physical and chemical changes in starch molecules, affecting their solubility, crystallinity, and molecular weight distribution, as cold plasma treatments significantly enhance starches' solubility and paste clarity by inducing depolymerization and reducing

AC (Chauhan, Kalaivendan, Eazhumalai, & Annapure, 2023). Also, these treatments increase the helical order of amylose, making it suitable for high-temperature food applications (Sifuentes-Nieves et al., 2021). Moreover, cold plasma treatments can narrow the molecular weight distribution of amylose, as seen in studies involving sequential treatments with RF plasma and electron beam irradiation. This narrowing is attributed to random chain scission and molecular rearrangements induced by the treatments (Braşoveanu et al., 2024). Gupta, Guha, and Srivastav (2023) noted the reduction in AC and changes in molecular weight distribution in taro starch, where cold plasma treatment led to significant changes in solubility and paste clarity, suggesting potential applications in food packaging and pharmaceuticals.

2.3.3. Film-forming ability

Amylose's film-forming ability is exploited in producing biodegradable packaging materials. In addition, amylose-based films are flexible, transparent, and have good gas barrier properties, making them suitable for packaging foods and pharmaceuticals as an eco-friendly alternative to synthetic plastics (Zou et al., 2021). Also, pre-gelatinized high-amylose starch films exhibit superior elongation compared to films made from normal corn starch due to improved starch dispersity and higher AC. This flexibility is crucial for maintaining close contact with food surfaces, which can enhance the sensory experience by providing a more uniform texture (Xi et al., 2024). The fractionation of amylose from tapioca starch has shown that films with higher AC possess better tensile strength and elongation properties. This results in films that are stronger and more pliable, contributing to a more pleasant mouthfeel when consumed. The morphology of amylose-based films, characterized by a smooth surface without pores or cracks, contributes to a visually appealing product. The smoothness and uniformity of the film surface can enhance the sensory perception of quality and purity (Sondari et al., 2022). Besides, the hydrophobicity of amylose films affects their interaction with moisture and other substances. A moderate contact angle suggests a balance between hydrophilic and hydrophobic properties, which can influence the film's appearance and how it feels to the touch (Sondari et al., 2022). On the other hand, AICs can encapsulate volatile compounds, potentially enhancing flavor and aroma retention in edible films. This encapsulation can protect sensitive ingredients from degradation, thereby preserving the intended sensory profile of the food product (Liu et al., 2023). However, the mechanical properties of amylose films can be enhanced by blending with other biopolymers or incorporating plasticizers. So, recent research focuses on improving the strength and flexibility of amylose films to expand their applications in various industries (Kumari & Sit, 2023).

3. Differentiating features of amylose

The attributes of starch are shaped by its structure, specifically the distribution of amylopectin branch chains and the amount of amylose, determining its properties and applications (Guo et al., 2022). The starch composition usually falls within the 50–80 % range, varying by plant source. Approximately 20–30 % of this composition consists of linear and slightly branched amylose, while 70–80 % comprises highly branched amylopectin (Al-Maqtari et al., 2023). Hence, amylose can accommodate various molecules, making it suitable for various food, medicinal, pharmaceutical, and industrial applications. This section provides a concise overview of the significance of amylose in several fields. Table 2 presents the distinguishing characteristics of amylose in food, medicine, pharmaceuticals, and nutrition fields.

3.1. Distinguishing characteristics in the food industry

Amylose has significant implications in various domains, especially in the food industry, and its levels vary significantly depending on a specific application. It is an important factor in starch-rich foods, as it can impact the texture and mouthfeel of the food and its subsequent applications, including food labeling, food quality control and optimization, and food and culinary innovation (Obadi et al., 2023). So, the determination of AC is a key and essential step in studying the structural characteristics and functional properties of starch (Mauro, Vela, & Ronda, 2023). Starch with higher AC tends to form firmer gels when heated (Liu et al., 2021), which is desirable in some food products like custards, puddings, gelled desserts, fillings, and sauces. Similarly, high-amylose starches, like those found in wheat, maize, barley, and potato, exhibit enhanced nutritional values and functional properties, resisting enzymatic degradation during heat treatment and forming structures that aid in digestive resistance (Li, Gidley, & Dhital, 2019). In food products like sausages and processed meats, amylose can serve as a binding agent, helping to hold ingredients together and improve the overall texture and consistency. Amylose is also an effective thickening agent, and proper AC can help thicken soups, sauces, and gravies, providing the desired consistency and viscosity to these culinary preparations (Himashree, Sengar, & Sunil, 2022). Besides, amylose contributes fewer calories per gram compared to fats, making it a beneficial ingredient in low-calorie or reduced-fat food products. The potential of amylose to form ICs with guest molecules has also sparked attention in the food sector, where it may be employed to regulate the release of bioactive compounds (Di Marco et al., 2022). Moreover, amylose can bind with lipids to form A-LC, affecting starch properties, digestibility, and health implications, making it a valuable component for creating resistant starch with potential health benefits (Huang et al., 2020). These compounds also considerably affect starch properties such as retrogradation, rheological properties, gelatinization properties, and solubility (Huang et al., 2020). Likewise, amylose can form ICs with hydrophobic molecules, making it a valuable tool for creating delivery systems for sensitive bioactive compounds and flavors, protecting them during processing, storage, and digestion (Di Marco et al., 2022). Additionally, amylose's fine structure in potato flakes influences oil uptake during the production of deep-fried crisps, impacting caloric density and texture (Reyniers et al., 2020). Furthermore, amylose can be utilized to encapsulate sensitive compounds like vitamin D, enhancing their stability and release properties, thus improving bioaccessibility in functional foods (Liu et al., 2023). In addition, amylose is being investigated for its potential application in developing biodegradable films and packaging materials, owing to its exceptional capacity to produce films and its propensity to undergo biodegradation (Deng et al., 2024). However, amylose's versatility in forming complexes and modifying starch properties makes it a crucial ingredient in developing functional foods with improved nutritional value and industrial scalability. So, appropriate AC can help generate the desired texture, stability, and overall quality of numerous food products, to a great extent, which makes amylose an essential ingredient in a wide range of culinary and processed food applications, benefiting both the sensory and nutritional aspects of food. Thus, it can be said that understanding and controlling AC helps to create diverse food products catering to consumer preferences and nutritional needs.

3.2. Medical and pharmaceutical industrial

Amylose plays a crucial role in modern medicine, where amylose forms ICs with various guest molecules, including lipids and flavor compounds, leading to the creation of resistant starches that resist digestion in the small intestine and impact postprandial blood glucose and insulin levels (Wheller et al., 2023). Foods high in amylose, such as whole grains and legumes, are associated with slower and more sustained increases in blood sugar levels (C. Chen et al., 2022). Furthermore, studies have investigated the effect of amylose on the digestion of starch-lauric acid-β-lactoglobulin protein complexes, and the results have identified amylose as an important structural factor that affects the kinetics and mechanism of digestion (Li et al., 2023). In the context of amyloidosis, amylose is a complication of chronic inflammation that predominantly affects the kidneys, with histological evaluation of renal

Table 2
Differentiating features of amylose in the fields of food, medical, pharmaceutical, and nutritional.

Type	Application	Contributing features	Summary	Reference
High-amylose starch	Food, medical, pharmaceutical, and nutritional industries	The molecular and microstructural features of high-amylose starch contribute to digestive enzyme resistance, while the functional properties and nutritional values support food applications	High-amylose starch offers unique functions and nutritional value to food products and maintains their structure during heat treatment, further enhancing their resistance to digestion	(Li, Gidley, et al., 2019)
A-LC	The food and pharmaceutical field	A-LC can develop nanocapsules for drug delivery. It also affects the solubility of starch, which affects its breakdown in the body.	A-LC offers potential health benefits, influencing glycemic levels and potentially reducing colon cancer risk. These complexes have potential applications in nanoencapsulation for drug delivery.	(Huang et al., 2020)
Amylose inclusion complexes	Functional foods	Amylose inclusion complexes can act as dietary fiber and protective delivery systems for bioactive compounds within food products.	The amylose complexes included show potential for use in functional foods, enhancing nutritional and functional value and serving as delivery systems for bioactive compounds.	(Di Marco et al., 2022)
V-amylose	Pharmaceutical field	V-amylose presents as a promising drug delivery vehicle due to its superior encapsulation properties, resistance to degradation, High-Loading Efficiency, and ability to control the release of therapeutic agents.	V-amylose's helical shape encapsulates medications, especially amphiphilic or hydrophobic compounds, and protects them from enzyme breakdown during bodily transport. V-amylose also allows efficient loading of high concentrations of therapeutic molecules and regulated drug release, allowing sustained therapeutic effects and tailored distribution to specific body parts.	(Prasher, Fatima, & Sharma, 2021)
V-amylose	Pharmaceutical field	Amylose can act as a protective carrier for ulcerative nonsteroidal anti-inflammatory drugs within the gastrointestinal tract and potentially provide a controlled release.	Amylose can have controlled release and enhanced bioavailability due to Its unique structure, biocompatibility, and ability to form drug complexes, allowing for a more targeted and sustained delivery to the site of action.	(Fatima et al., 2024)
Amylose-dicarboxylic acid inclusion complexes	Functional foods	Amylose-dicarboxylic acid inclusion complexes' high thermal stability and V-type helical structures offer potential for innovative food and industrial applications.	Amylose forms inclusion complexes with long-chain dicarboxylic acids, influencing starch properties with high thermal stability and V-type helical structures with high dissociation temperatures.	(Kenar et al., 2022)
Anionic and Ampholytic high-amylose starch	Pharmaceutical field	Aqueous modification of high-amylose starch derivatives produced multifunctional excipients with potential applications in drug delivery.	The modified derivatives exhibited decreased crystallinity, blue value, density, and viscosity compared to unmodified high-amylose starch. This suggests a lower organized structure, altered helices, and increased solubility.	(Labelle et al., 2023)
High amylose-based biocomposites	Food and pharmaceutical field.	High-amylose starch offers unique properties that enhance the nutritional value of food products and make has a promising material for eco-friendly biomedical applications.	The unique features of high-amylose starch make it appropriate for developing ecologically friendly products, boosting food nutrition, biodegradable films and other biomaterials, and environmental friendliness.	(Faisal et al., 2022)
Amylose-lipid powder complexes	Functional foods	Spray-dried amylose-lipid powder complexes had smaller particles, lower bulk densities, poorer digestion rates, and high-resistant starch. These powders may be useful in functional foods that regulate blood sugar or enhance fullness.	Spray-drying amylose-lipid powder complexes, especially at higher temperatures, may cause structural alterations that promote resistant starch production. This resistant starch is harder to break down by digestive enzymes, slowing glucose release and impacting overall digestion behavior.	(Yun, Devahastin, & Chiewchan, 2020)
Nano-helices of amylose	Food, medicine, pharmaceuticals, and nutrition	Amylose nano-helices are biodegradable and can be used in food, medicine, pharmaceuticals, and nutrition.	V-amylose nano-helices form two polymorphs. So, V-amylose ligand networks offer a promising novel food component encapsulation strategy and potentially more controlled method for delivering bioactive food.	(Rostamabadi, Falsafi, & Jafari, 2019)
Amylose inclusion complex	Functional foods	Encapsulating vitamin D in an amylose complex enhances its stability, release, bioaccessibility, and protection from light and oxygen degradation, making it better for food fortification.	The amylose molecules form a cage-like structure around the vitamin D molecules, offering protection, high encapsulation efficiency, and high loading capacity. Amylose encapsulation improves vitamin D stability and release, making it a better functional food option.	(Liu et al., 2023)
High-amylose maize flour	Food field	The biscuits formulated with high-amylose maize flour regulate satiety-related sensations and potentially reduce food intake.	The AC in the biscuits might be responsible for the observed effects. Amylose impacts glycemic and insulin responses after meals, potentially influencing feelings of fullness (satiety).	(Giuberti et al., 2021)
High-amylose Rice	Food field	The rice containing 19–25 % amylose offers a desirable texture and eating quality while maintaining health benefits (slower digestion).	The molecular features of starch play a significant role in determining the cooked texture and preference for rice. Hardness was identified as a key factor influencing palatability, with softer rice generally preferred.	(Tao, Yu, Prakash, & Gilbert, 2019)
High amylose corn starch (HACS)	Functional foods	The greater resistant starch content of HACS could be used for gradual and regulated glucose release.	HACS has better structural integrity, which may explain its favorable connection with gelatinization enthalpy. Structure stability may delay digestive enzymes, increasing resistant starch.	(Lv, Hong, Zhou, & Jiang, 2021)

amyloid deposits serving as a prognostic tool for renal function and survival outcomes (Buob et al., 2022). In imaging, bone scintigraphy using a radiopharmaceutical made of a bisphosphonate linked to amylose has shown great sensitivity in identifying bone abnormalities, making it useful in the early stages of disease (Delcroix, Le Pennec, Salaün, & Querellou-Lefranc, 2023). Additionally, amylose derivatives have been utilized as chiral selectors in capillary electrophoresis for separating racemic drugs efficiently, showcasing the versatility and significance of amylose in diverse medical applications (Yu & Quirino, 2019).

On the other hand, amylose-based nanosystems have emerged as a major advancement with the potential to revolutionize drug delivery methods. Amylose is a strong candidate for drug encapsulation material due to its low swelling tendency, minimal steric hindrance, flexibility, enhanced tensile strength, and superior water resistance compared to native starch (Di Marco et al., 2022). Moreover, amylose-based delivery methods are designed for targeted release in the colon. This is because they can create films with a microstructure through gelation. These films are resistant to the effects of pancreatic α-amylase but can be digested by the amylases produced by the microbiota in the colon (Prasher et al., 2024). In addition, when exposed to gastrointestinal conditions, the amylose-based coatings have the capacity to expand and create small openings to release drug molecules. This property is used in the development of enteric coatings for solid dosage forms (Labelle et al., 2023).

3.3. Nutritional advantages of amylose

Amylose is essential in multiple physiological processes that lead to human well-being, and diets with a higher proportion of amylose-rich foods are often considered healthier due to their slower digestion and potential benefits for weight management and blood sugar control (Costabile et al., 2023). Amylose significantly affects the glycemic index (GI) of foods, which is one of its most noteworthy advantages (Kunyanee & Luangsakul, 2020). A-LC provides health benefits, including influencing insulin and glycemic levels and lowering the incidence of colon cancer. They can also be employed to nanoencapsulate bioactive or sensitive compounds (Huang et al., 2020). Recent research has indicated that foods with a high AC generally have a lower GI in comparison to those that are abundant in amylopectin (Fitriani, Dieny, Margawati, & Jauharany, 2021), as the foods that have a high AC lead to a delayed process of digestion and a gradual release of glucose into the bloodstream. This attribute is especially advantageous for persons who have insulin resistance or diabetes, as it aids in the more efficient regulation of blood sugar levels. The slower rate of digestion of amylose not only aids in the regulation of blood sugar but also enhances the sensation of fullness or satiety (Giuberti, Albertini, Miggiano, Dall'Asta, Rossi, 2021). This phenomenon can be explained by the fact that foods high in amylose undergo a slower process of digestion in the digestive system, resulting in extended signals of feeling full and satisfied (Li, Dhital, & Gidley, 2022). As a result, people may consume fewer calories in total, which helps in managing weight and preventing obesity. Lim et al. (2021) conducted a study showing that mice fed a high-amylose wheat diet experienced increased feelings of fullness and decreased levels of hunger compared to those who followed a diet high in amylopectin. Amylose also has a crucial function in promoting gastrointestinal well-being. It evades digestion in the small intestine and largely remains intact as it reaches the colon, and it acts as a base for helpful gut bacteria, stimulating the creation of short-chain fatty acids (SCFAs) like butyrate (Xie et al., 2021). SCFAs are crucial for preserving colon health, diminishing inflammation, and potentially decreasing the likelihood of developing colorectal cancer. Li, Zhang, Zhu, Chao, and Guo (2023) emphasized in a recent review that amylose has a favorable impact on the gut microbiota by promoting the growth of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*. The alterations in the makeup of the gut microbiota are linked to enhanced digestion, improved immunological function, and enhanced overall gut health. Consuming amylose has been associated with a decreased likelihood of developing several chronic illnesses. The combination of a low glycemic response, satiety promotion, and gut health support reduces the likelihood of acquiring type 2 diabetes, cardiovascular illnesses, and some types of cancer.

4. AC analysis methods

Understanding AC is vital for assessing food nutritional properties and developing starch-based products with desired characteristics like texture, viscosity, and stability (Shen et al., 2019). Various methods exist for determining AC, each with unique advantages, limitations, and applications. Techniques range from image processing with artificial neural networks (ANN) (Saputra, Wijayanto, Ristiyana, Purnamasari, & Muhlison, 2022) to association mapping with SSR and SNP markers (Sa, Park, Jang, & Lee, 2023), digital image photometry using smartphones (Tuaño, Castrillo, & Viola, 2021), and near-infrared spectroscopy (Xie et al., 2022). Advancements aim to enhance accuracy, efficiency, and usability. However, several analytical methods available for qualitative and quantitative determination of amylose in starch or starchy products have been collected and presented in this review (Fig. 3), the choice of which depends on the precision level and equipment availability. All the methods for determining AC are summarized in Table 3.

4.1. Qualitative and semi-quantitative methods

Qualitative and semi-quantitative methods for AC determination differ in the level of detail and precision they provide. Qualitative methods, like the iodine test, are simpler and faster, ideal for initial screening. Semi-quantitative methods, such as spectrophotometry, provide a better idea of AC but lack the precision of quantitative methods. The choice depends on the analysis goals and required accuracy.

4.1.1. Iodine staining (IS)

Although the IS method for determining AC is not a recent innovation, it remains relevant and valuable in various fields due to its simplicity and effectiveness. The IS method is a widely used qualitative or semi-quantitative technique for assessing AC. This method relies on the interaction of amylose with iodine ions, forming a blue complex, while amylopectin remains colorless. Amylose, a long spiral structure formed by α-glucose molecules (Kim & Jung, 2022), exhibits color changes (from colorless to blue) with an increased DP in a certain range. Although IS qualitatively assesses relative AC in starch samples through color intensity, it lacks precise quantitative measurement. However, recent studies showed that the IS method was used to screen crop varieties with desired AC in agricultural breeding, which is crucial for developing crops that meet specific nutritional and processing requirements. AC-highly related food quality evaluation and control based on the IS method have also been implemented to ensure the desired texture and consistency of final products, including rice beer (Park, Park, Chung, & Oh, 2023), noodles (Yang, Dhital, Zhang, Wang, & Chen, 2022), and edible film (Zou et al., 2021). As part of nutritional studies, AC assessment of functional foods using the IS method helped assess the impact of different foods on blood sugar levels and overall dietary quality. Despite its limitations, IS remains a fast and convenient method for visually evaluating AC, making it suitable for research, education, and preliminary screening of starch samples. It is widely used for comparing AC in different samples or assessing the presence or absence of amylose. However, sample complexity, including lipids and proteins, can interfere with color changes and affect results (Reddappa et al., 2022).

4.1.2. Clarity test (CT)

CT, commonly known as the hot water test, is a simple test that uses amylose's hazy or opaque suspension and amylopectin's clear solution in hot water (Reddappa et al., 2022). Amylose is present when a starch

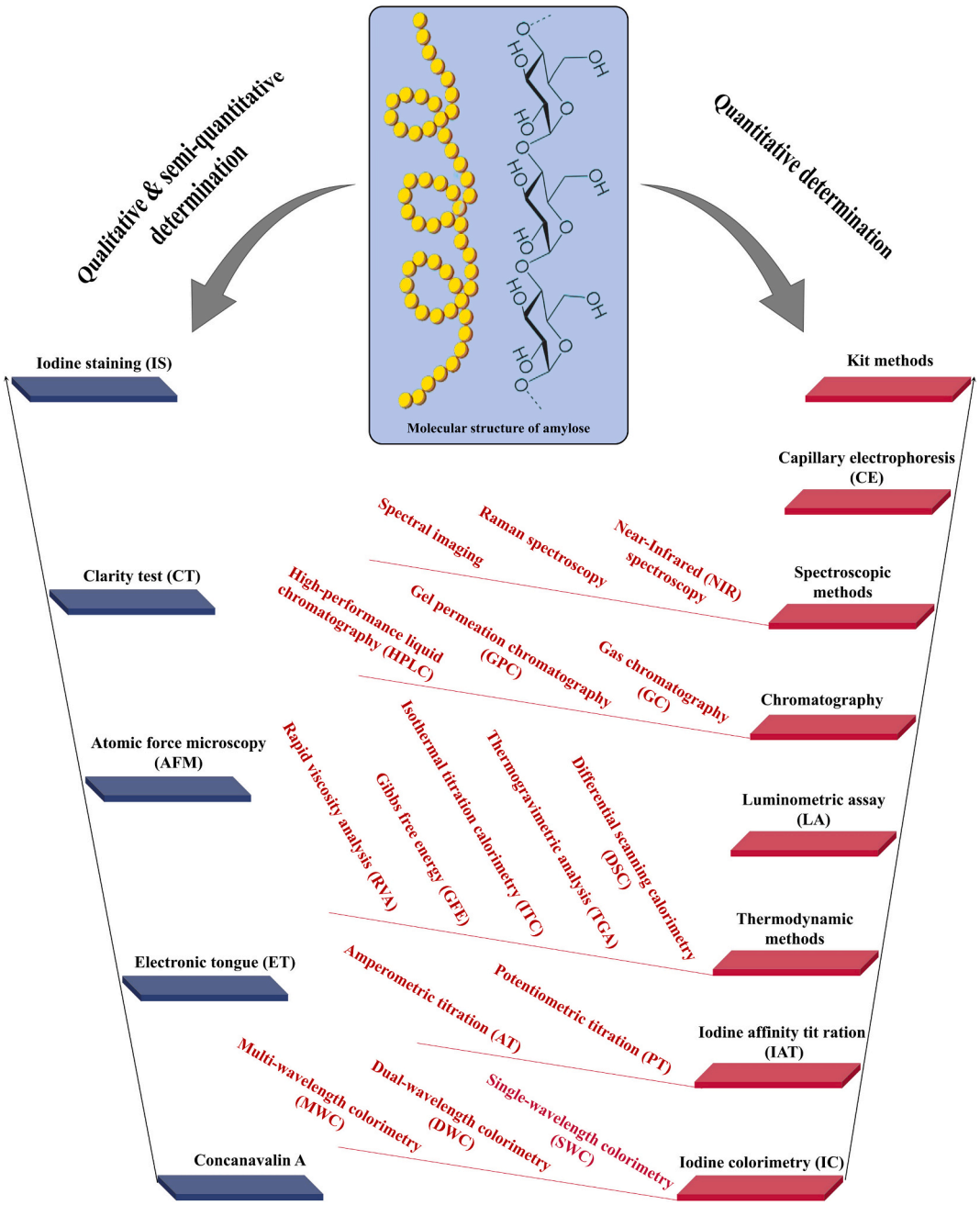

Fig. 3. Various methods for determining AC.

sample combines thoroughly with hot water and remains cloudy or opaque. CT is unsuitable for precise quantification, making it useful for educational purposes and quick initial assessments of AC in starch samples. CT was used to assess starch paste clarity in potato cultivars (Karmakar, Miya, Chakraborty, & Roy, 2022), jack beans (Akinyemi, Orishadipe, Ebun-Oluwa, & Aladesanmi, 2020), and others. Although CT has certain historic uses, it is less frequently utilized or acknowledged than other amylose identification procedures like IS, chromatography, or spectroscopy. Recently, analytical technology has made AC detection more accurate and efficient, decreasing the need for CT. However, CT can still be utilized for starch analysis in cases with acceptable limitations.

4.1.3. Atomic force microscopy (AFM)

AFM is a powerful high-resolution imaging technique that utilizes a sharp probe to scan the surface to visualize and characterize the surface properties of starch granules in a sample (Deka, Gautam, Duarah, Roy, & Dutta, 2024). The AFM probe interacts with starch samples to provide visible topography representations of their exterior morphology, rheology, and interactions at a micro- or nano-structural level. AFM has been used to quantify and visualize amylose granule sizes and shapes and characterize their microstructural, mechanical, and textural features in starch-based foods (Wen, Xu, Liu, Corke, & Sui, 2020). AFM was used to study the nanoscale granule shapes, gelation properties, and interactions of amylose-containing starch in wheat (Li et al., 2022) and some fruits or vegetables (Huang et al., 2023). AFM is qualitative and rarely utilized for AC quantification. AFM's high-resolution imaging and nanoscale analytical capabilities make it useful for studying starches and amylose-based materials' morphological properties, helping food scientists, materials scientists, and pharmaceuticals understand amylose behaviors.

Table 3
Comparison of different methods to measure AC in terms of advantages, limitations, and applicability.

Method		Advantages	Disadvantages/ Limitations	Applicabilities
IS		1) Relatively quick 2) Qualitative or semi-quantitative 3) High sensitivity 4) Visual detection 5) Versatility	1) Calibration with known standards or reference samples is required 2) Interferences from other compounds in samples 3) Relative measurements 4) Dependent on the purity and homogeneity of the samples	• Used for determining AC and analyzing starch properties • Quality control of AC in starch-based foods • Comparative studies of samples with different AC or AC changes • Initial screening to estimate AC
CT		1) Simple and easy to perform 2) Qualitative or semi-quantitative measurement 3) Rapid and inexpensive estimation	1) Interferences from other compounds in samples 2) Lack of precision 3) Subjective 4) Limited accuracy	• Be valuable for educational purposes • Rough screening of starch samples with high AC or low AC • Initial assessment of AC in samples
AFM		1) Qualitative or semi-quantitative 2) Non-destructive 3) High resolution 4) Surface morphology characterization	1) Sample preparation required 2) Slow imaging 3) Primarily for surface analysis 4) Not precise quantitative measurements	• It is commonly used to visualize and characterize amylose granules and study their properties. • Valuable for morphological studies of amylose granules
ET		1) Qualitative or semi-quantitative 2) Non-destructive 3) Rapid analysis 4) High sensitivity	1) Calibration and training required 2) Qualitative or semi-quantitative 3) Limited specificity 4) Not precise quantitative measurements 5) Dependent on the sample type	• Assess and control the AC or other taste-related attributes of starch-based products • valuable for sensory analysis of taste profiles associated with AC
Concanavalin A		1) Simple 2) Rapid 3) Specific binding to amylose	1) Interferences from specific binding 2) Sensitivity to sample conditions 3) Qualitative or semi-quantitative 4) Special reagents need	• Qualitative or semi-quantitative analysis of AC in starch samples • Screening applications where a quick assessment of AC is needed
IC	SWC	1) Simple and easy to implement 2) Rapid detection 3) Quantitative measurement 4) A label-free technique	1) Interferences from other compounds in samples 2) Limited specificity 3) Lack of accuracy 4) Highly dependent on sample purity	• Rapid screening of samples with AC • Initial assessments of the presence or absence of amylose • Suitable when specificity is less critical and rough estimation of AC required
	DWC	1) Direct measurement 2) Quantitative measurement 3) Improved specificity 4) High sensitivity 5) Relatively rapid and easy to perform 6) A label-free technique	1) Proper sample preparation is needed 2) Susceptible to interferences from other compounds in samples	• Determination of AC in starch samples • Quality control of AC in starch-based foods • Comparative studies of samples with different AC or AC changes • Routine analysis of AC
	MWC	1) Direct measurement 2) Quantitative measurement 3) High sensitivity 4) Relatively rapid and easy to perform 5) A label-free technique	1) Proper sample preparation is needed. 2) Susceptible to interferences from other compounds in samples 3) Limited specificity	• Determination of AC in starch samples • Quality control of AC in starch-based foods • Comparative studies of samples with different AC or AC changes • Routine analysis of AC
IAT	PT	1) Direct measurement 2) Quantitative measurement 3) High sensitivity 4) A label-free technique	1) Time-consuming 2) Complex procedures needed 3) Skilled personnel required 4) Highly dependent on sample purity 5) Electrode maintenance required	• Precise determination of AC in starch samples • Quality control of AC in starch-based foods • Comparative studies of samples with different AC or AC changes • Particularly useful for sensitive analysis of low-level AC detection
	AT	1) Direct measurement 2) Quantitative measurement 3) High accuracy in AC quantification 4) A label-free technique	1) Time-consuming 2) Complex procedures needed 3) Skilled personnel required 4) Highly dependent on sample purity 5) Accurate interpretation of the titration curve requires expertise	• Precise determination of AC in starch samples • Quality control of AC in starch-based foods • Comparative studies of samples with different AC or AC changes • Used for standardized detection of AC
Thermodynamic methods	DSC	1) Direct measurement 2) Quantitative measurement 3) A label-free technique 4) Providing thermodynamic insights insight into the energetics of the amylose gelatinization process 5) Real-time information on heat flow during thermal transitions of amylose-containing materials	1) Calibration with known standards or reference samples is required 2) Proper sample preparation is needed 3) Complex interpretation of DSC data 4) Influenced by sample complexity 5) Limited to relative information for AC	• Characterization of amylose gelatinization • Quality control of AC in starch-based foods • Comparative studies on the thermal behavior of samples with different AC • Thermal stability investigation of amylose-containing materials
	TGA	1) Direct measurement 2) Quantitative measurement 3) A label-free technique 4) Real-time monitoring of weight loss or degradation of amylose	1) Calibration with known standards or reference samples is required 2) Proper sample preparation is needed 3) Limited to relative information on AC 4) Complex interpretation of TGA data 5) Influenced by sample complexity	• Characterization of thermal degradation of amylose • Quality control of AC in starch-based foods • Comparative studies on the thermal behavior of samples with different AC • Thermal stability investigation of amylose-containing materials

(*continued on next page*)

Table 3 (*continued*)

Method		Advantages	Disadvantages/ Limitations	Applicabilities
	ITC	1) Direct measurement 2) Quantitative measurement 3) Providing thermodynamic insight into the energetics of the amylose-iodine binding process 4) A label-free technique 5) Real-time monitoring of binding interactions	1) Specialized equipment required 2) The large sample size required 3) Complex data analysis and interpretation 4) Influenced by sample purity 5) Limited to binding studies of amylose-iodine 6) Limited to relative information for the amylose-iodine binding process	• Investigation of thermodynamics and kinetics of amylose-iodine binding interactions • Comparative studies of binding affinities of samples with different AC • To reveal the amylose-iodine binding mechanism
	GFE	1) Quantitative measurement 2) Thermodynamic insight and profiling of amylose-iodine interaction 3) Relative comparisons between samples containing different AC	1) Indirect measurement 2) Specialized equipment required 3) Computational complexity and Data analysis complexity 4) Influenced by sample variability 5) Limited to relative information for the amylose-iodine binding process	• Understanding the thermodynamics of the amylose-iodine interaction • Comparative analysis of amylose-iodine interactions • Assessment of the stability of amylose-iodine complexes • Fundamental insights into amylose behavior through molecular interactions
	RVA	1) Rapid & convenient 2) Small sample size 3) Multiple parameters 4) Realistic simulation of conditions 5) Easy to operate	1) Indirect measurement 2) Calibration required 3) Data complexity and result variability 4) Limited accuracy and lack of precision	• Assessment of the starches' suitability for various applications • Quality control of AC in starch-based foods • Investigation of the properties of starch from different sources • AC estimation • Rapid screening of starch samples
LA		1) Quantitative measurement 2) Rapid detection 3) High sensitivity 4) Versatility	1) Calibration with known standards or reference samples is required 2) Influenced by other compounds in samples 3) Relative measurements 4) Dependent on sample purity and homogeneity	• Determination of AC in starch samples • Quality control of AC in starch-based foods • Comparative studies of samples with different AC or AC changes • Initial screening of samples with different AC
Chromatography	GC	1) Effectively distinguish between amylose and amylopectin 2) High precision 3) High sensitivity	1) Specialized equipment required 2) Proper sample preparation is required 3) chemical derivatization required 4) Relative measurement	• Quantitative assessment of AC • Quality control of AC in starch-based foods • Comparative studies of samples with different AC or AC changes
	GPC	1) Effectively distinguish between amylose and amylopectin 2) High precision 3) Applicable to various starch samples	1) Calibration with standards or reference samples is required 2) Proper sample preparation is required 3) Specialized equipment required 4) Relative measurement	• Quantitative assessment of AC • Quality control of AC in starch-based foods • Comparative studies of samples with different AC or AC changes
	HPLC	1) Accurately distinguish between amylose and amylopectin 2) High precision 3) Wide applicability	1) Specialized equipment required 2) Proper sample preparation and solubilization required 3) Relative measurement	• Quantitative assessment of AC • Quality control of AC starch-based foods • Comparative studies of samples with different AC or AC changes
	NIR	1) Rapid and non-invasive 2) Reagent- and pollution-free 3) Quantitative measurement 4) Multi-component analysis 5) Wide applicability	1) Calibration model required 2) Complex data analysis 3) Calibration model transfer 4) Sample homogeneity	• Rapid quantitative assessment of AC • Quality control of AC in starch-based foods • On-line process monitoring
Spectroscopic methods	Raman	1) Rapid and non-invasive 2) Reagent- and pollution-free 3) Quantitative measurement 4) High specificity 5) Minimal sample preparation	1) Fluorescence interference 2) Affected by sample variability 3) Calibration required	• Rapid quantitative assessment of AC • Quality control of AC in starch-based foods • Analysis of AC at the microscopic level
	Spectral imaging	1) Rapid and non-invasive 2) Reagent- and pollution-free 3) Quantitative measurement 4) Multi-component analysis 5) Visualization	1) Calibration model required 2) Complex data analysis 3) High equipment cost 4) Interference from sample heterogeneity and environmental conditions	• Studying AC, distribution, and molecular structures • Quality control of AC starch-based foods • Visualizing the spatial distribution of amylose
CE		1) Minimal Sample Requirements 2) Quantitative measurement 3) High separation effect 4) Rapid analysis	1) Proper sample preparation and solubilization required 2) Specialized equipment required 3) Calibration with standard AC is required	• Rapid quantitative assessment of AC • studying starch properties • Quality control of AC starch-based foods
Kits		1) Convenient 2) Time-saving 3) Consistency 4) Reduced risk of errors 5) Reproducibility 6) Standard included 7) Accurate and reliable	1) High cost 2) Kit components vary from different manufacturers 3) Environmental implications of generated waste 4) Limited application samples	• Rapid quantitative assessment of AC • Quality control of AC starch-based foods • Studying starch characteristics

4.1.4. Electronic tongue (ET)

ET is an analytical taste simulator. The system uses non-specific, low-selectivity chemical sensors to detect taste-related substances in samples using pattern recognition (Wesoły, Przewodowski, & Ciosek-Skibińska, 2023). ET can measure amylose by detecting taste-related chemicals associated with AC in solutions or food products such as rice and corn (Deore, Sonar, Kasabale, & Wetal, 2019). It is often used for qualitative or semi-quantitative analysis and is suitable for quality control and sensory analysis applications in the food industry (Marx, 2023). However, the limited specificity and the need for calibration and training should be considered when applying ET for amylose determination.

4.1.5. Concanavalin A (ConA)

ConA is a type of protein used to specifically bind to certain carbohydrate molecules, having a high affinity with α-D-glucose and α-D-mannose residues (China, Dohi, & Kumar, 2023), which are present in linear amylose rather than in branched amylopectin. In the assessment of AC, ConA serves as a reagent to bind to amylose molecules selectively, allowing for qualitative or semi-quantitative determination of AC in starch samples (Seung, 2020). The ConA method was used to investigate the structural and physicochemical properties of starches from Korean rice cultivars with different AC (Park, Oh, Chung, & Park, 2020). The effect of the amylose-amylopectin ratio on rice noodle quality was also studied by measuring AC using ConA (Zhang et al., 2022). Other recent reports on AC determination by ConA further allowed for a deeper insight into the specific evaluation of the physicochemical and structural properties of various starches (Chen & Zhu, 2024). Therefore, ConA is more suitable for applications where a rapid assessment of AC is sufficient but cannot provide precise quantitative measurements. Although ConA has the advantages of specificity and simplicity, it may exhibit a certain affinity for other carbohydrates, affecting the accuracy of the results.

4.2. Quantitative methods

Accurate analysis of starch qualities in different applications, such as food science and medicines, requires using quantitative methodologies to determine AC. These methods include image processing techniques and ANN model (Saputra et al., 2022). Another method utilizes digital image photometry (DIP) with a smartphone camera and free-access software (Tuaño et al., 2021). Combining ion exchange and gel filtration chromatographic methods is another method (Arunachallam, Kumaravel, & Gopal, 2023). Near-infrared (NIR) spectroscopy has also been used as a non-destructive method for determining AC (Fan et al., 2022). These methods offer precise, consistent, comprehensive measurements crucial for research and industry purposes. However, this section will briefly discuss quantitative methods for determining AC in different samples.

4.2.1. Iodine colorimetric (IC) methods

IC methods are currently the most essential and classical AC measure. The approach uses a spectrophotometer to measure the light absorption of the blue-purple amylose-iodine complex at specified wavelengths and construct a linear relationship between AC and absorbance to determine AC in unknown starch samples. The intensity of the complex color change is directly proportional to the AC in the sample; samples with higher AC exhibit greater absorbance. The IC method is simpler and cheaper than the IS test and can be used for routine amylose analysis in food and agriculture research and quality control. It also determines AC more accurately and quantitatively.

One method that falls under the IC methods is single-wavelength colorimetric (SWC), a simplified and quick method for determining AC in starch samples. It uses the absorbance of a starch-iodine complex at a specific wavelength (usually 620 or 638 nm) and is suitable for qualitative or approximate quantitative assessments (Wang et al., 2022). The AC of the starch sample can be calculated by comparing its wavelength absorbance to the standard curve. The SWC method was recently applied to measure AC to evaluate the nutritional and processing quality of starch-containing products. Some fruit seeds as alternatives to natural starch have also been studied through AC analysis using the SWC method (Yaowiwat, Madmusa, & Yimsuwan, 2023).

Dual-wavelength colorimetric (DWC) is a more advanced and precise method for determining AC in starch. DWC uses the differential absorbance qualities of amylose and amylopectin at two wavelengths (620 and 550 nm) to determine AC in starch more accurately than SWC. DWC has better selectivity and sensitivity than SWC; however, sample preparation and correlation curves of standard amylose and amylopectin samples with iodine solution are still required. The latest research on natural starches, modified starches, and starchy products used the DWC method to determine AC in multifunctional composites (Mei et al., 2023).

Another method is multi-wavelength colorimetry (MWC), which requires using three or more wavelengths to calculate AC in starch. AC determination typically uses 535, 620, or 730 nm wavelengths. By measuring absorbances at several wavelengths and calculating ratios or differences, the AC in a starch sample may be reliably measured with great precision. MWC is a preferred choice for research, quality control, comparative studies, and analytical applications requiring precise amylose quantification, like amylose-related gene control (Wang et al., 2022). However, the MWC method requires many wavelengths and more complicated calculations. Proper sample preparation and potential interferences should also be considered to ensure an accurate determination.

Overall, colorimetric methods offer sensitivity and high throughput, making them suitable for analyzing multiple samples; however, interference from contaminants and amylopectin can affect accuracy (Saputra et al., 2022).

4.2.2. Iodine affinity titration (IAT) methods

Iodine affinity is used to measure AC and amylose purity in starch. Each starch has a unique iodine affinity value based on its AC (Dewan, Shobhit, & Kajla, 2024). High AC shows greater iodine affinity. When titrated with iodine, a starch-iodine combination does not affect solution voltage or current in the IAT method. During titration, linear branch chains like amylose form a blue-glow helical combination with iodine molecules. Starch's apparent AC increases iodine affinity (Singh, Singh, & Saxena, 2021). The quantities of the starch-iodide complex and AC are determined by linearly fitting the curve of voltage or current changes to the iodine titration curve. Potentiometric titration (PT) and amperometric titration (AT) are the specific methods used for this analysis.

The PT assay measures voltage changes during sodium thiosulfate titration to quantify AC in a starch sample. When the potential stabilizes, amylose and iodine react completely. Relative AC (as a percentage of sample weight) is calculated from sodium thiosulfate solution volume consumed during titration. PT-measured amylose levels have increased interest in starch-containing composite production and attributes (Feng, Wang, Campanella, Zhang, & Miao, 2023; Zhivkov, Popov, & Hristova, 2023). However, PT measures AC accurately and efficiently, but it requires careful sample preparation and competent staff. Similarly, the AT method quantifies AC by electrochemically reducing iodine by amylose (Pandiselvam, Gomathy, Kothakota, Zehla, & Mayookha, 2019). The titration procedure and the subsequent calculation process are similar to PT. This approach has measured AC in high-protein starch sources. Although the IAT method has the advantages of specificity and simplicity, free iodine ions will be generated, which will cause the solution voltage or current to change when the iodine solution is used in excess.

4.2.3. Thermodynamic methods

Thermodynamic methods for determining AC in starch samples involve measuring various thermodynamic parameters associated with amylose-amylopectin interactions, such as enthalpy, entropy, viscosity,

and free energy. Differential scanning calorimetry (DSC) is a widely used technique for determining AC in starch, relying on the distinct thermal properties of amylose and amylopectin (Shi, Zhao, Qin, Liu, & Wang, 2023). Amylose interacts with lysophosphatidylcholine to form complex compounds, which, when heated, generate a heat absorption peak proportional to AC (Pandiselvam et al., 2019). DSC is valuable in food research and quality control for various starch-containing products, allowing for the investigation of structural changes in starches with different ACs (Pan et al., 2022). Additionally, the thermal properties of various plant starches with differing ACs can be evaluated using DSC (Zhao et al., 2023). Despite its simplicity, speed, accuracy, and automation advantages, DSC requires expensive, specialized instruments.

Thermogravimetric analysis (TGA) is another analytical technique that can measure changes in starch mass with temperature fluctuations to determine AC (Lemos, Barbosa, Ramos, Coelho, & Druzian, 2019). This method relies on the differential weight loss during the thermal decomposition of amylose and amylopectin. Amylose shows a specific temperature range for thermal degradation, leading to weight loss. The AC is quantified by comparing this weight loss to a known starch standard. TGA is a valuable technique for characterizing starches' thermal properties and decomposition behavior in food research applications but requires careful sample preparation and calibration for accurate AC quantification (Feng et al., 2024).

Isothermal titration calorimetry (ITC) is a precise analytical technique to determine thermodynamic interactions between molecules in a solution (Bastos et al., 2023). ITC measures the enthalpy change during the binding interaction between amylose and iodine, aiding in amylose quantification (Yu et al., 2021). While ITC offers valuable insights into amylose-iodine interaction thermodynamics, it requires expertise and specialized equipment, making it more suitable for research, quality control, and comparative studies than routine use in the food and starch industries (Falconer, Schuur, & Mittermaier, 2021).

Similarly, Gibbs free energy (GFE) is a thermodynamic parameter that describes a chemical reaction's spontaneity and direction (Abell & Bretz, 2019). The Gibbs free energy change (ΔG) for the amylose-iodine binding reaction is calculated to determine AC (X. Chen et al., 2022). ΔG is related to the enthalpy change (ΔH) and entropy change (ΔS) of the reaction, calculated as $\Delta G = \Delta H - T\Delta S$, where T is the absolute temperature in Kelvin (Yu et al., 2021). This calculation provides insights into the energetics and stability of the amylose-iodine interaction, aiding the characterization of amylose-containing systems. However, GFE is an indirect method and is not typically used for direct quantitative determination of AC in samples.

Rapid viscosity analysis (RVA) is essential for evaluating starch quality and predicting its performance as a food component (Tian et al., 2022). During a heat-hold-cool-hold cycle, RVA assesses the gelatinization, gelling, and retrogradation processes of starch, which are influenced by its structure (Zhong et al., 2022). Although not a direct method for determining AC, RVA analyzes the rheological properties of starch suspensions, using viscosity profiles and calibration curves to estimate AC indirectly. It offers simple operation, environmental control, small sample size, fast detection, and temperature control. High amylose-containing starches' contributions to food pasting properties were rapidly analyzed using RVA (Tian et al., 2023). Recent studies have used RVA to investigate starch structures and digestibility with different ACs (Li et al., 2023; No & Shin, 2023). While RVA provides AC estimates, it should be complemented with other techniques for precise and quantitative measurements.

4.2.4. Luminometric assay (LA)

The LA employs chemiluminescence, a process where light is emitted as a result of a chemical reaction, to measure AC in different samples. This method involves an optical sensor array that detects minute amounts of chemiluminescence by obtaining differential outputs from selected pixels, enhancing sensitivity and accuracy. The LA, also called the amylose/amylopectin ratio assay, is an enzymic tool for AC determination that is based on a detectable luminescent signal produced in the interaction between the oxidase/peroxidase reagent and glucose released from the enzymatic hydrolysis of amylopectin. Amylose is less branched and more resistant to enzymatic hydrolysis compared to amylopectin. As a result, the amount of glucose released is directly proportional to the amylopectin content, while the luminescence detected is inversely proportional to the AC in the sample (Farhana et al., 2021). LA is a sensitive and relatively quick method for estimating AC in starch samples, but it is not commonly used. However, proper calibration and potential interferences are required in the LA method to ensure accurate AC determination. LA can be used as an initial choice to assess AC in starch samples before more precise quantification.

4.2.5. Chromatography

Chromatography techniques are widely used for analyzing AC. High-performance size-exclusion chromatography (HPSEC) with refractive index detection is a popular method for studying molecular characteristics such as AC, molar mass, and molecular weight (Pandiselvam et al., 2019). Agarose gel column chromatography is another technique that separates and quantifies high-amylose starch components, including amylose, intermediate components, and amylopectin. Additionally, chiral stationary phases (CSPs) in HPLC are used for the chiral resolution of compounds, including chiral drugs (Rizzo, Benincori, Fontana, Pasini, & Cirilli, 2022). These CSPs are effective for compounds with different stereogenic elements like central, axial, helical, and planar stereogenicity.

In contrast, gas chromatography (GC) provides precise and sensitive AC determination in starch via molecular-size separation and derivatization. GC separates starch compounds based on molecular size and volatility (Zhao, Smyth, Tao, Henry, & Gilbert, 2022). This involves converting starch into volatile derivatives, vaporizing them, and injecting them into a GC column. Amylose, being smaller and less branched, elutes faster than amylopectin. Recent studies have used GC for AC determination in amylose-other substance complexes (Sun et al., 2023). However, GC is a relative method requiring specialized equipment, expertise, and calibration to ensure accuracy.

On the other hand, gel permeation chromatography (GPC), also known as size exclusion chromatography (SEC), accurately determines AC in starch by separating molecules based on size (Szwengiel & Kubiak, 2020). SEC in an aqueous medium is termed gel filtration chromatography (GFC), while in an organic solvent, it is called GPC. Due to starch chains' low stability in neutral aqueous solutions, accurate molar mass determinations can be challenging. In GPC, the sample dissolves in a solvent and passes through a column with porous beads, separating amylopectin and amylose based on their molecular weights. GPC is useful for starch research and quality control (Suzuki, Hanashiro, & Fujita, 2023), and there is a good correlation between AC values from the IS method and GPC. GPC analysis helps investigate the molecular weight distribution and structural characteristics of starches from various sources. However, GPC requires trained professionals for accurate implementation.

HPLC is also a precise quantitative method for determining AC in starch samples by separating molecules based on molecular weight (Pandiselvam et al., 2019). In HPLC, starch samples are dissolved, injected, and eluted, with amylose separating from amylopectin due to its smaller size. Chiral stationary phases (CSPs), like amylose tris (3-chloro-5-methylphenylcarbamate) and amylose (3,5-dimethylphenylcarbamate), have proven effective for chiral separations (Cao et al., 2021; Rizzo et al., 2022). The effects of AC on food products have been investigated via HPLC, which also revealed AC's impact on V-type granular starch formation (Zhou, He, & Jin, 2024). HPLC provides relative AC measurements but requires skilled personnel and specialized equipment. Chromatography provides valuable insights into the content and characteristics of amylose for various applications.

4.2.6. Spectroscopic methods

NIR spectroscopy is a rapid, non-destructive method used to determine AC in starch samples based on the interaction between molecules and NIR light (Diaz, Kawamura, Matsuo, Kato, & Koseki, 2019). Amylose and amylopectin exhibit distinct spectral signatures in the NIR region, facilitating their differentiation. NIR spectroscopy measures NIR light absorption, reflectance, or transmittance in a sample, employing chemometrics to analyze spectral data and correlate it with AC values. NIR has been extensively studied for AC determination in various cereal foods, such as wheat, rice, millet, sorghum, maize, buckwheat, barley, and hulless oat, demonstrating strong correlations (determination coefficient (R2), > 0.90) with AC values (Olsen et al., 2021). NIR, as a non-contact technique combined with different chemometrics, has also been applied to detect AC in cereal grains like rice (C. Chen et al., 2022; Cheng et al., 2023) and various root tubers (Adesokan, Alamu, Otegbayo, & Maziya-Dixon, 2023). Despite its advantages of speed, non-invasiveness, and simultaneous analysis of multiple components, NIR requires calibration models and expertise in data analysis, with ongoing efforts to improve its precision in analyzing AC in diverse products compared to standard methods.

Raman spectroscopy is another technique used in the food science and industry to determine AC. It offers sensitivity to molecule structures and their environments, making it valuable for non-contact selective probing (Pezzotti et al., 2021). For example, despite sharing glucose ring units, differences between amylose and amylopectin highlight the Raman probe's sensitivity to structural variations. Raman spectroscopy, utilizing inelastic scattering of laser light, is non-destructive and specific in assessing AC in starch samples (Chen, Zhang, Wang, & Wang, 2023). It distinguishes amylose and amylopectin due to their unique Raman spectra and benefits from minimal sample preparation and high spatial resolution (Pezzotti et al., 2021). This technique has successfully quantified AC in rice and shows promise for amylose screening in various samples. However, fluorescence correction and calibration models are necessary for accurate AC determination using Raman spectroscopy.

Another method is spectral imaging, which combines traditional imaging with spectroscopy, capturing images across multiple wavelengths (spectral bands) in the electromagnetic spectrum, including visible and NIR regions. Each pixel contains spectral information, enabling analysis of AC properties. Spectral imaging, including hyperspectral imaging with high spectral resolution, has proven valuable for the quality evaluation of various foods, particularly for AC analysis (Xu et al., 2023). Spectral imaging is effective for amylose studies in starch-based materials but demands specialized data processing and calibration for quantitative analysis.

4.2.7. Capillary electrophoresis (CE)

CE is a separation technique involving charged molecule migration in an electric field through a narrow capillary filled with electrolytes (Kalaycıoğlu & Erim, 2022). CE determines amylose and amylopectin in starch samples based on weight, electric charge, and electrophoretic mobility differences. Amylose quantification involves analyzing migration times or peak areas in the electropherogram (Aredes et al., 2023). CE has been used to separate and quantify amylose in starch samples from various botanical origins, achieving a low detection limit (0.1 mg/mL) and faster detection compared to previous methods. Recent CE studies examined amylose chain-length distribution effects on long-term rice starch kinetics (Li, Hu, & Li, 2021). Fluorophore-assisted CE characterizes AC in different starches or starchy foods (Song, Deng, Zhang, Ren, & Zhao, 2023). CE is an alternative to HPLC for AC analysis in biological tissues, offering high resolution, quantitative capabilities, and rapid analysis, though it requires specialized equipment and electrophoresis expertise.

4.2.8. Kit methods

A kit method, typically referring to an assay or technique that is packaged and provided with ready-to-use reagents, materials, and instructions, simplifies specific analytical or diagnostic tests (Pandiselvam et al., 2019). Kit methods vary in content and design, tailored to different analyses. For instance, the Amylose/Amylopectin Assay Kit is widely used for AC measurement based on enzymatic reactions, suitable for amylose determination in cereal starches, flours, pure starches, and foods (Taddei, Galassi, Nocente, & Gazza, 2021; Waleed et al., 2021). This method offers convenience, consistency, accessibility, reproducibility, and reduced error risk in AC analysis, albeit at a cost.

5. Trends and prospects for future improvement

The determination of AC is of significant value in various industries, including food, agriculture, pharmaceuticals, and biotechnology (Boetje et al., 2023). Researchers and industries are constantly seeking more efficient, accurate, and cost-effective methods for amylose determination. This section proposes and summarizes the future trends and prospects of the above methods for detecting and quantifying amylose.

5.1. Chromatographic techniques

Chromatographic techniques are pivotal in amylose determination and could best develop through some improvements. Key areas of improvement include: 1) Enhanced resolution and sensitivity: Focused efforts are needed to improve the resolution and sensitivity of chromatographic methods. This involves refining sample separation and detecting lower amylose concentrations. Improved sample preparation methods, chromatographic columns, and detectors will contribute to the precision of amylose determination. 2) Integration with mass spectrometry: The integration of chromatography with MS enhances the identification and quantification of amylose, facilitating superior structural characterization and discrimination from other carbohydrates. 3) Advances in data analysis: Ongoing advancements in data processing tools, such as chemometrics and AI, are elevating the precision and efficiency of amylose quantification and characterization through chromatographic analysis. 4) Miniaturization and high-throughput analysis: The rising popularity of miniaturized chromatographic systems and high-throughput techniques enables faster analysis and reduced solvent consumption. Miniaturized systems with automatic detection enhance efficiency, making them suitable for routine quality control of amylose-based samples. 5) Eco-friendly practices: Chromatographic methods are evolving to prioritize eco-sustainability, accountability, and education by minimizing hazardous reagent usage and waste generation. Sustainable chromatography practices align with global efforts to reduce the environmental impact of analytical chemistry. In summary, integrating mass spectrometry and advanced data analysis methods propels chromatographic techniques for amylose determination, promising improved resolution, sensitivity, and sustainability.

5.2. Spectroscopic techniques

Spectroscopic techniques, including NIR spectroscopy, Raman spectroscopy, and spectral imaging, have undergone significant advancements in amylose determination and are poised for continued evolution. Key trends and prospects include: 1) Enhanced sensitivity and resolution: Ongoing research aims to boost sensitivity and resolution in spectroscopic methods, allowing for the detection of lower amylose levels and finer structural details. This will broaden the applicability and accuracy of these methods in trace analysis and starch-based material research. 2) Integrated chemometric analysis: Chemometric techniques, such as machine learning (ML) and artificial intelligence (AI), are increasingly integrated to extract valuable information from spectroscopic data, enhancing the accuracy and speed of amylose quantification. 3) Non-invasive analysis: Spectroscopic methods are trending towards non-invasive or minimally invasive analysis, particularly in

biomedical and pharmaceutical applications. Non-invasive spectroscopy can offer insights into amylose determination in biological tissues and pharmaceutical formulations without sample destruction. 4) Multispectral imaging: Multispectral imaging techniques provide specific spectral signatures for amylose identification by capturing images at multiple wavelengths. This is expected to facilitate quantitative amylose determination and characterization in complex samples. 5) Instrument miniaturization and portable devices: ongoing efforts to miniaturize spectroscopic instruments and develop portable devices enable on-site, real-time amylose determination in various industries, including food and agriculture. Portable spectrometers equipped with advanced data analysis software streamline amylose analysis in remote or field settings. 6) Customization for specific applications: Spectroscopic techniques are becoming more customizable and optimized for specific applications, such as monitoring amylose in agri-food products or assessing amylose in pharmaceutical formulations. This customization ensures efficient and reliable amylose determination, tailored to the unique requirements of different industries. Overall, spectroscopic techniques are evolving to provide higher sensitivity, portability, and versatility in amylose determination. The integration of advanced data analysis methods, coupled with the rise of multispectral imaging and equipment miniaturization, promises comprehensive insights into amylose research across various industries.

5.3. Microscopic imaging

Microscopic imaging techniques, such as integrated microscopy and imaging, are rapidly advancing to enable the study of amylose distribution within starch granules at micro- or nanoscale levels. Key developments in these techniques promise enhanced insights into amylose structure and distribution. The major trends and prospects in this field include the following: 1) Improved resolution and sensitivity. Microscopic imaging is being continuously advanced and oriented towards enhancing spatial resolution and sensitivity, allowing for the visualization of amylose at the nanoscale. Higher-resolution microscopy will provide more detailed information about amylose granules, facilitating precise quantification and structural analysis. 2) Three-dimensional (3D) imaging and tomography. 3D imaging and tomography techniques are becoming more accessible, enabling the visualization of amylose in native 3D space. 3D imaging will provide a better understanding of the spatial distribution and interactions of amylose within complex matrices. 3) Fluorescence and labeling techniques. The use of fluorescent probes and labeling techniques is increasing, enabling the specific and targeted visualization of amylose in biological and food samples. Fluorescence-based imaging methods will play a significant role in tracking amylose dynamics and interactions in various contexts. 4) Multimodal imaging. Combinations of different microscopy modalities, such as fluorescence, confocal, and electron microscopy, are gaining popularity for comprehensive amylose analysis. Multimodal imaging offers complementary information about amylose structure and distribution, enhancing the accuracy and depth of amylose analysis in starch-containing samples. 5) AI for image analysis. Integrating AI and ML in image analysis can enhance the automated quantification and classification of amylose. AI-driven image analysis will improve the speed and accuracy of amylose determination, making it more efficient and accessible. Microscopic imaging techniques are evolving and will offer improved resolution, sensitivity, and versatility for amylose determination. They are trending to promote advancements in the visualization and analysis of amylose and benefit research, quality control, and applications across diverse industries.

5.4. Biosensors and electronic tongues

Biosensors and electronic tongues are emerging technologies with great potential for amylose determination and analysis and can be innovated in some aspects, including: 1) Specialized biosensors for amylose determination: Biosensors are often characterized by high specificity, rapid response, and the potential for real-time monitoring of amylose levels. The design and development of specialized biosensors for amylose detection is gaining momentum. These biosensors can utilize biological recognition elements like enzymes, antibodies, or aptamers immobilized on the sensor's surface to achieve rapid amylose identification. Paper-based biosensors are becoming popular due to their simplicity, cost-effectiveness, and ease of use. Further development of paper-based biosensors may result in portable and disposable devices suitable for amylose determination in resource-limited settings. As technology advances, these dedicated biosensors may become valuable tools for amylose analysis, food quality control, and other applications. 2) Nanomaterials and nanotechnology: Integrating nanomaterials such as nanowires or nanoparticles into biosensor platforms improves detection performance by increasing sensitivity and signal-to-noise ratios. Continuous advancements in nanotechnology may lead to more sensitive and selective biosensors for amylose determination, which offer potential capabilities to detect amylose at low concentrations. 3) Integrate into the Internet of Things (IoT): Biosensors can be integrated into IoT and be part of the whole IoT system, enabling remote monitoring and data transmission, which allows users to access real-time amylose data through connected devices. IoT integrated with biosensors holds promise for continuous and remote monitoring of amylose in food supply chains, achieving timely quality control and management. 4) Electronic tongues for complex mixtures: Electronic tongues, consisting of arrays of sensors that mimic human taste and smell senses, are being explored for assessing complex carbohydrates, including amylose. The evolving electronic tongues will provide valuable insights into the taste and quality of food products containing amylose-rich ingredients. In brief, biosensors and electronic tongues show great promise for amylose determination across various applications. Continued research and technological innovation may lead to commercializing biosensors and electronic tongues for routine amylose analysis.

5.5. Enzymatic methods

Enzymatic methods for amylose determination have a rich history and are witnessing continuous advancements with promising trends. Key developments include: 1) Automation and high-throughput screening: Integrating automation and robotic systems enhances enzymatic assays, facilitating high-throughput screening for amylose in various samples. This automation accelerates analysis processes, making these methods suitable for medium- to large-scale applications. 2) Enzyme modification: Ongoing research explores novel techniques to modify enzymes for specific amylose-related applications, allowing for the customization of enzymes. Tailored enzymes with improved catalytic properties expand the versatility of enzymatic methods. 3) Enhanced enzymes and assay kits: efforts focus on improving the specificity, sensitivity, and stability of enzymes used in amylose determination. Commercial assay kits featuring optimized enzymic reagents have gained popularity, promising more accurate and reliable amylose quantification, particularly in routine analyses and quality control of starch-based foods. 4) Customized assays for specific matrices: Developing tailored enzymatic assays is a growing trend, valuable for meeting specific requirements in sample matrices such as food products and biological tissues. Customized assays enhance the accuracy of amylose determination in complex samples. 5) Miniaturization and portable testing: Miniaturizing enzymatic assays facilitates the development of portable or handheld devices, enabling on-site amylose analysis in fields like food, healthcare, and agriculture. 6) Regulatory compliance: Regulatory agencies increasingly emphasize accurate amylose determination, particularly in the food and pharmaceutical sectors. This drives the need for validated enzymatic methods to meet regulatory requirements essential for quality control and compliance statements. So, enzymatic methods for amylose determination are evolving to provide improved specificity, sensitivity, and efficiency. Automation, miniaturization, and

sustainability influence trends, emphasizing customization and versatility in these assays and shaping the future of enzymatic amylose determination in research and industry applications.

5.6. Combined methods

Compared to a single technology, integrating multiple analytical techniques is also a good option to offer comprehensive insights into AC and structure. Combining multiple analytical techniques can be performed to obtain complementary information about amylose, which will provide a holistic view of amylose, enabling more accurate determination and structural characterization, for example: 1) Combination of chromatography and spectroscopy: The integration of chromatographic and spectroscopic methods may allow for simultaneous amylose quantification and structural analysis, enhancing the accuracy of amylose determination and providing valuable insights into amylose properties. 2) Spectroscopy and microscopy synergy: Combining microscopy with spectroscopic techniques, such as Raman or NIR, may offer high-resolution images of amylose granules with detailed chemical information, which will facilitate the amylose composition and structural characterization comprehensively. 3) Integration of advanced imaging and enzymic analysis: Integration of advanced imaging, such as hyperspectral imaging or MS, with enzyme-based analytical methods may expand the capabilities of the combined approaches, which will provide abundant data for amylose detection, making imaging techniques more versatile and informative.

6. Conclusion

The structural and functional properties of amylose are fundamental to its diverse applications. Its ability to form helical complexes, interact with other molecules, and enhance food texture and stability makes it a versatile biopolymer. Advances in analytical methods, such as thermodynamic analysis, HPLC, spectroscopic methods, and enzymatic assays, have improved our understanding of amylose. As research progresses, amylose's potential to develop sustainable materials, improve health outcomes, and create innovative food products will expand. Future trends in amylose determination should focus on improving accuracy, speed, and ease of use. These advancements will promote further research, quality control, and applications across various industries, deepening our understanding of amylose and its roles. Ongoing innovations in analytical techniques and data analysis will make amylose determination more accessible and efficient, with increasing research highlighting its significance in food, medical, and other industrial sectors.

CRediT authorship contribution statement

Yuling Wang: Writing – original draft, Validation, Software. **Xingqi Ou:** Writing – original draft, Validation, Investigation, Formal analysis. **Qais Ali Al-Maqtari:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Investigation, Data curation, Conceptualization. **Hong-Ju He:** Writing – review & editing, Supervision, Software, Funding acquisition. **Norzila Othman:** Investigation, Writing – review & editing.

Declaration of competing interest

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

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data availability

Data will be made available on request.

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