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Review Article

The molecular mechanisms and new classification of resistant starch – A review

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ABSTRACT

Diabetes is one of the major chronic diseases endangering human health. Because of the low glycemic index, RS (resistant starch) plays an important role in the intervention and treatment of diabetes. Based on our previous studies, the mechanism of RS is systematically discussed from the molecular level (the relationship between starch molecular conformation and RS, the relationship between double-helix structure and RS, and the relationship between molecular complexation and RS) and granular level (the relationship between amylase penetration and RS, the relationship between crystallinity and RS, and the relationship between amylose/amylopectin content and RS). In addition, with more in-depth studies in the preparation and formation reason of RS, the current classification of RS may not satisfy our current understanding of RS. In this paper, a new classification of RS is proposed. RS is classified into 10 types from two aspects (reparation methods and formation reasons), which may make it easier to understand the different RS. This paper may provide a reference for the research of RS.

1. Introduction

Diabetes is one of the major chronic diseases endangering human health. According to a survey by the International Diabetes Federation (IDF), as of 2025, there are about 540 million patients with diabetes worldwide. By 2045, IDF projections show that 1 in 8 adults, approximately 783 million, will be living with diabetes (A survey by the International Diabetes Federation (IDF and)). According to the different enzymatic hydrolysis rates, starch can be divided into RS (resistant starch), SDS (slowly digestible starch), and RDS (rapidly digestible starch). RDS refers to the starch that can be completely digested within 20 min, RS refers to the starch that can not be digested within 120 min. Because of the low glycemic index, RS plays an important auxiliary role in the management of diabetes(Wang et al., 2023).

Currently, RS is categorized into five types: RS-1 (physically inaccessible starch), RS-2 (resistant granules), RS-3 (retrograded starch), RS-4 (physically or chemically modified starch) and RS-5 (starch-lipid complexes) (Dobranowski and Stintzi, 2021). At present, RS mainly comes from: (1) Natural resistant starches such as brown rice and quinoa, which have an outer layer of fiber that can impede digestion. (2) Regenerated starch, which is the main method of RS preparation in practical applications. However, starch retrogradation will lead to moisture loss and reduced elasticity, which resulted in a significant reduction in product quality. (3) High amylose starch, this is also one of the most common RS currently on the market. (4) Various modified starches and RS5, which are mainly from experimental studies.

In this paper, the molecular mechanism of resistant starch is systematically discussed based on previous studies, which may provide an important reference for the research and preparation of RS. Meanwhile, it may provide a reference for other studies discussing RS formation mechanisms. In addition, a new classification of RS is proposed to replace the existing one, which may make the theoretical system of resistant starch more complete.

2. Starch hydrolysis

2.1. The essence of starch hydrolysis

The essence of starch hydrolysis is that the amylase nucleophilically attacks the glycosidic bonds of starch to break it down into

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monosaccharides, disaccharides or oligosaccharides(Lin et al., 2023). Here we take human α-amylase as an example. As shown in Fig. 1, human α-amylase is a protease consisting of 8 α-helices and 14 β-sheets, with an Rg value of 3.23 nm, a specific surface area of 193.7 nm^2, a molecular weight of 55 kD, and a pI of 6.87. As shown in Fig. 1a and b, an active crack with a length of approximately 3.5 nm and width of approximately 1.5 nm exists on the surface of α-amylase, and is responsible for binding starch chains. An active groove with a depth of approximately 1.4 nm is located in the middle of the active crack. The catalytic triad (Asp197-Glu233-Asp300) is located at the bottom of the active groove(Zhong et al., 2024a). Glu233 releases protons and attacks the glycosidic oxides in the catalytic triad. Asp197 is responsible for the formation and stabilisation of carbon-ion intermediates. Asp300 is responsible for stabilizing the nucleophilic reagent (water molecule) and helps Glu233 extract protons from the water molecule to produce activated hydroxyl ions, and finally the hydroxyl ions attack the carbon ion intermediate to complete the reaction. In addition, six flexible loops (Only flexible loop2 and a few amino acid residues in flexible loop3 are hydrophobic. The rest of the flexible loops and the bottoms of the active cracks are hydrophilic.) are located around the active crack, which are responsible for substrate recognition and binding (Zhang et al., 2022).

When a starch molecule interacts with α-amylase, as shown in Fig. 1c, the 8th and 9th glucose residues of the starch molecule are docked into the active groove of α-amylase, where the glycosidic bond

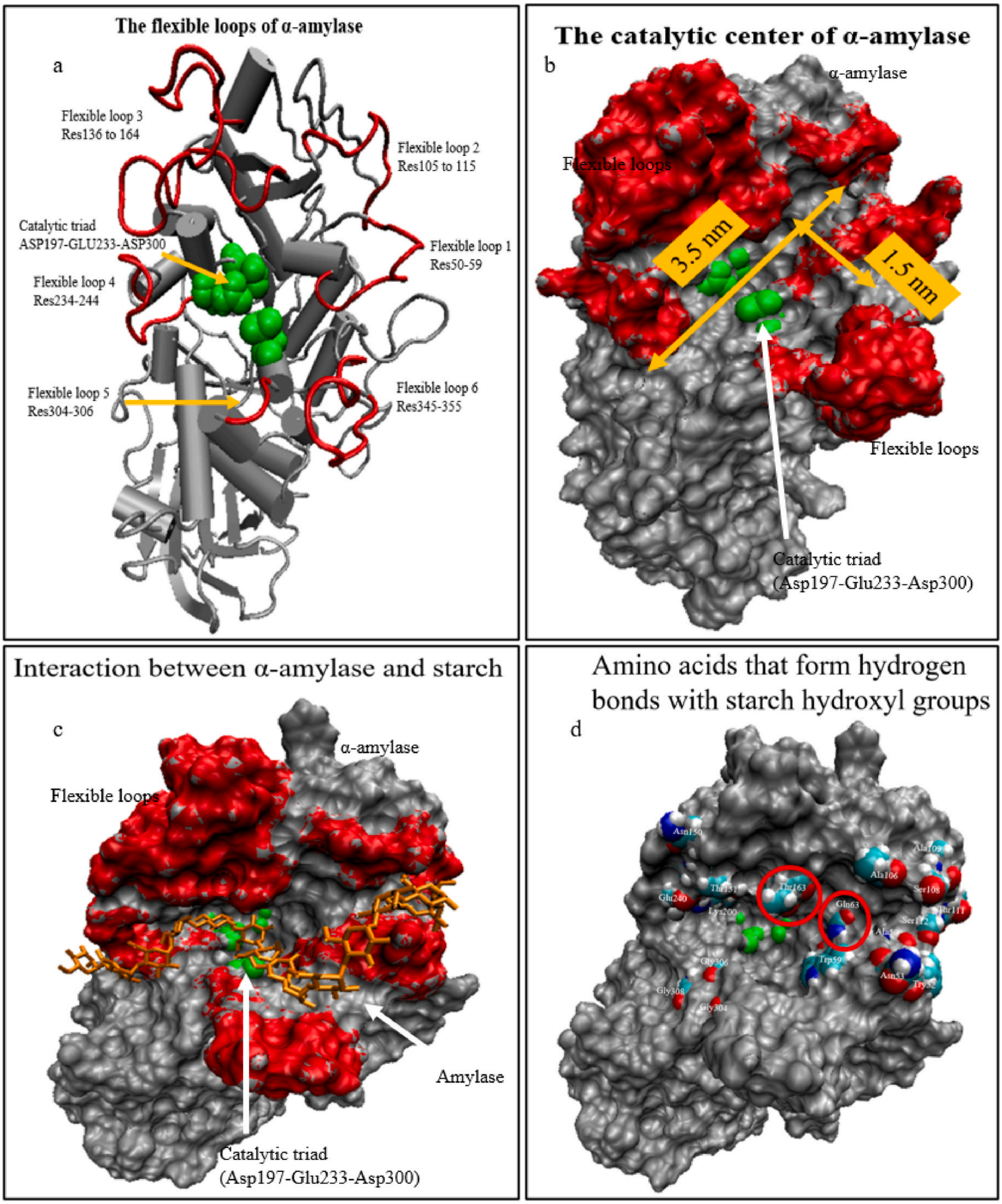

Fig. 1. The tertiary structural features of α-amylase and its interaction with starch molecule.

between the 8th and 9th glucose residues is closest to the catalytic triad. The distances between the glycosidic oxygen and carboxyl groups of Asp197, Glu233, and Asp300 are 0.47, 0.51, and 0.43 nm, respectively. And the head of the starch molecule is bound to the flexible loop3, 4, and the tail of the starch molecule bind to the flexible loop1, 2 of α-amylase (Zhong et al., 2024a).

Based on the calculation of the binding free energies, it can be seen that the total energy between starch molecule and α-amylase is about 78 kJ/mol, in which the van der Waals interactions account for more than 70% of the total energy. And the other 30% are electrostatic interactions and hydrophobic interactions. In addition, based on the polyhydroxy structure of starch, hydrogen bonding is an important non-bonding force in starch. The energy of hydrogen bonding is approximately 35 kJ/mol. As seen in Fig. 1d, the major residues that form hydrogen bonds with starch molecules are Thr163 and Gln63 (>60%), followed by Lys200, Try151, Asn53, Asn298, Asn105, and Ala307, which are of great significance for the hydrolysis of starch molecule (Zhong et al., 2024a).

2.2. The hydrolysis mode of α-amylase

As shown in Fig. S1, currently, there are two hypothesized hydrolysis modes of α-amylase: continuous sliding hydrolysis and discontinuous hydrolysis (The hydrolysis mode of β-amylase is considered to be continuous sliding hydrolysis(Wei et al., 2024)). Discontinuous hydrolysis refers to the detachment of the hydrolyzed starch chain from the amylase after the enzymatic hydrolysis of the starch chain, after which the enzyme re-recognises the starch molecule and re-hydrolyzes it (Koukiekolo et al., 2001). Continuous sliding hydrolysis means that once the starch chain is recognized and bound by the enzyme, it is no longer detached; rather, the starch chain slides and continues to be hydrolyzed through a conformational change (Oudjeriouat et al., 2003).

The hydrolysis mode of α-amylase is closely related to the hydrolysis rate, especially for RS5. The complexation of starch molecules may affect the sliding of starch molecular chains. However, there is still controversy about the hydrolysis mode of α-amylase. In a previous study, the interaction energy between the amylase-active groove and 6 glucose residues was calculated to be approximately 78 kJ/mol(Zhong et al., 2024a) (a C-C bond energy is about 320 kJ/mol). Based on this result it can be surmised that the starch molecular chains may not be easily detached from the amylase-active groove under this stronger interaction. In addition to this, the amino acid residues of α-amylase such as Thr163 and Gln63 at the active groove can also form hydrogen bonds with the hydroxyl groups of the starch molecule. Therefore, we believe that the more likely mode of hydrolysis is continuous sliding hydrolysis.

In summary, continuous sliding hydrolysis may be more consistent with the existing findings. However, it has not been fully proven. In the future, researchers may try to prepare short-chain starch molecules and prepare the crystal sample of starch-amylase complex. Then the hydrolysis mode of α-amylase may be directly observed by using cryo-electron microscopy.

3. The mechanisms of RS

3.1. Molecular lever

3.1.1. Relationship between starch molecular conformation and RS

The starch molecular conformation refers to the spatial arrangement of each group or atom in the starch molecule, including left-hand helix, V-helix, and irregular curls etc. Naturally, starch molecules tend to exist as left-hand helix conformation, as it has the lowest free energy and is the most stable conformation (Chen et al., 2020a). In our previous studies, it was found that starch molecular conformation would change significantly after different treatments by using molecular dynamics simulation. For example, after heating treatment, the number of the O2-O3 and O6-O6 hydrogen bonds between neighbouring glucose residues in the starch molecule decreases significantly, which makes starch molecule straight (Chen et al., 2022). And after high-pressure or salt treatment, the number of the O2-O3 hydrogen bond between neighbouring glucose residues increases significantly, which makes starch molecule to become bent(Zhong et al., 2022). In addition, these results from molecular simulation have been confirmed by using small angle X-ray diffraction and molecular sieve chromatography through calculating the Rg values(Zhong et al., 2024a).

In our previous study, the interactions between α-amylase and starch molecules with different conformations (left-hand conformation, straight conformation and bent conformation) were analyzed(Zhong et al., 2024a). It can be seen in Fig. 2, The interaction mode between starch molecules with different conformations and amylase is obviously different. The left-hand helix starch molecule can dock normally into the active groove of alpha α-amylase and is hydrolyzed from the middle of the starch molecule (See section 2.1 for details.). With respect to the bent conformational starch molecule, α-amylase can only recognize the glucose residues in the head of starch molecule and starts hydrolysis from the head of the starch molecule to produce monosaccharides directly, which is not conducive to the preparation of RS. The straight conformational starch molecule from heat treatment can also insert into the active groove of α-amylase, but can not penetrate deep enough to reach the correct site. The distances between the glycosidic oxygen of starch molecule and the carboxyl groups of Asp197, Glu233, and Asp300 are 0.82, 0.66, and 0.55 nm, respectively(Zhong et al., 2024a). This distance is not sufficient for nucleophilic attack of the catalytic triad (the distance for nucleophilic attack should be less than 0.5 nm(Goldbach et al., 2019)). Namely, α-amylase is unable to correctly recognize and hydrolyze this straight conformational starch molecule produced by heat treatment.

During heat treatment, the number of the O2-O3 and O6-O6 hydrogen bonds between neighbouring glucose residues in the starch molecule decreases significantly, after that starch molecules will transition from a low-energy left-hand helix conformation to a high-energy straight conformation(Chen et al., 2022). After heating treatment, as the temperature decreases, the high-energy straight conformation may spontaneously re-transition to the low-energy left-hand helix conformation. But this transition will take some time. Although researchers have not yet been able to clarify how much time this transition will take. However, at least within this time, the straight conformational starch molecule cannot be correctly recognized and hydrolyzed by α-amylase. Namely, this straight conformational molecule resulting from a significant reduction in the number of intramolecular hydrogen bonds is favourable for the preparation of RS. Therefore, in practice, breaking the intramolecular hydrogen bonds to make starch molecular conformation become straight is an important way to prepare RS.

3.1.2. Relationship between molecular complexation and RS

Food is a complex system. In addition to starch, various small molecule compounds such as lipids, proteins, small peptides and others are present in foods. In earlier studies, studies have found that, adding lipids to starch can reduce the rate of starch digestion. which is known as RS5 type resistant starch (Dobranowski and Stintzi, 2021).

In our previous study, the molecular mechanisms of RS5 has been analyzed(Zhong et al., 2024b). As shown in Fig. 3a–c, it can be seen that, the complexation of starch molecules with lipids leads to the emergence of a spatial site barrier, which prevents glucose residues complexed with lipids from penetrating completely to the bottom of the α-amylase active groove. The other two unentangled fatty acid chains of the triglyceride occupied the site of the α-amylase activity cleft. As shown in Fig. 3c, the distances between the glycosidic oxygen atom of the glycosidic bond nearest to the active centre and the carboxyl group on Asp197, Glu233, and Asp300 were 0.95, 0.77, and 0.65 nm, respectively, which could not guarantee a nucleophilic attack on the glycosidic bond by the catalytic triad of α-amylase(Ma et al., 2024). This is the fundamental reason for the formation of RS-5.

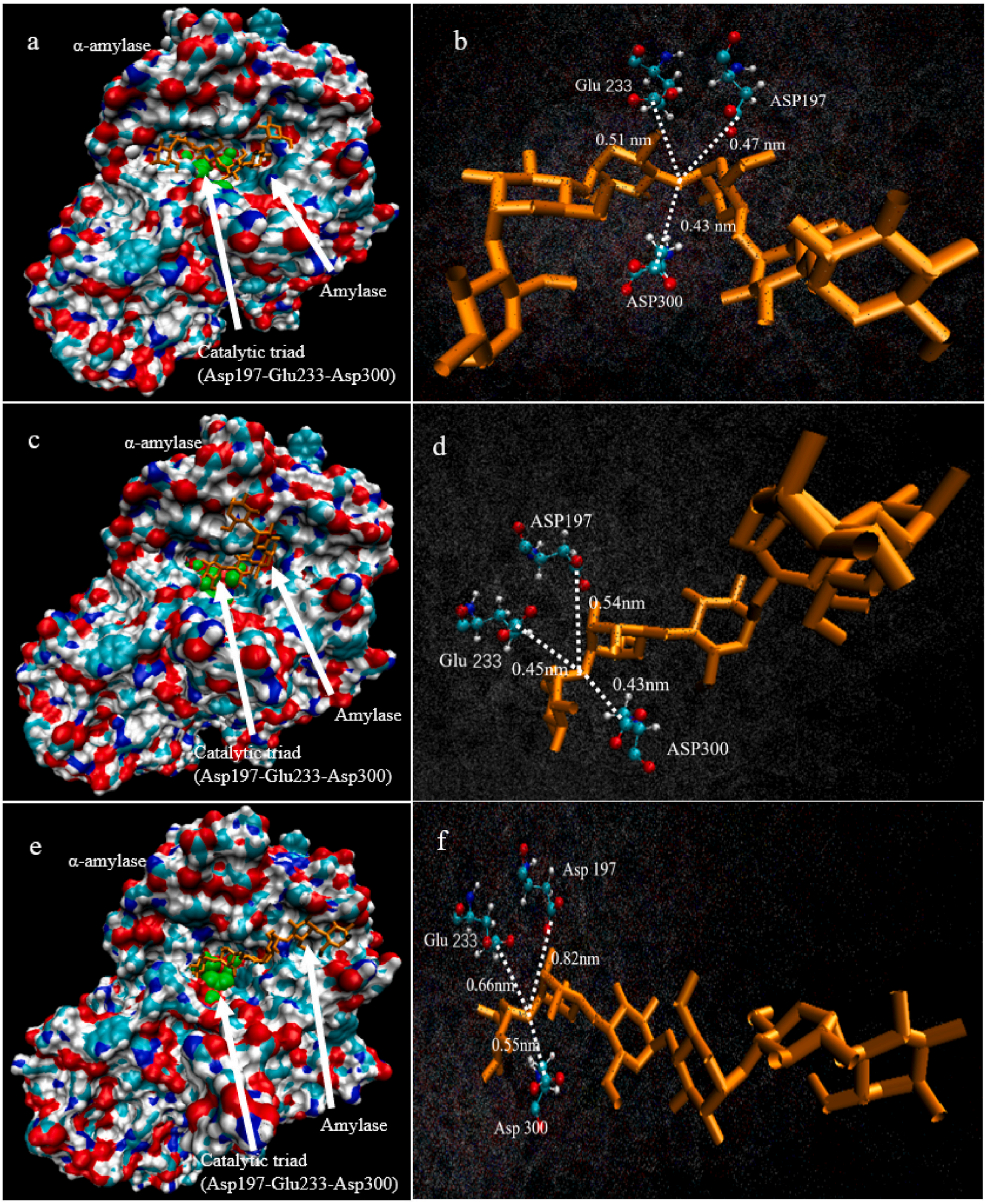

Fig. 2. Interactions between different starch molecules and α-amylase (a and b: left-hand conformation, c and d: bent conformation, e and f: straight conformation).

However, many researchers have found that the preparation of RS5 by lipid addition is not very efficient(Ma et al., 2024; Thakur et al., 2023; Tappiban et al., 2024). As shown in Figs. 3d to 4f, the glucose residues not involved in complexation were successfully docked to the active groove of α-amylase. Namely, these glucose residues not involved in complexation can be hydrolyzed normally by amylase. Besides, experiments have shown that, when lipids are complexed with starch, less than 20% of the glucose residues are directly involved in complexation, whereas more than 80% are not involved in complexation (Fig. 3g) (Chen et al., 2020b). This is the fundamental reason for the low efficiency of RS5 preparation. Therefore, it can be calculated that, the addition of 1 g of fat (molecular weight of triglyceride calculated at 650) would only turn approximately 2 g of starch into RS. However, when the amount of added fat exceeded the saturated oil absorption rate of starch (approximately 15%), the RS content could not be increased further. Assuming a saturated oil absorption rate of 15%, adding a sufficient amount of lipids to 100 g of starch will convert approximately 30 g of starch into RS.

Moreover, excessive lipid intake may be hazardous to human health. Thus, lipids may not be the best choice for the preparation of RS5. In our previous studies, as shown in Fig. 4, triglyceride molecules were replaced with small peptide and decanal molecules. And the starch-

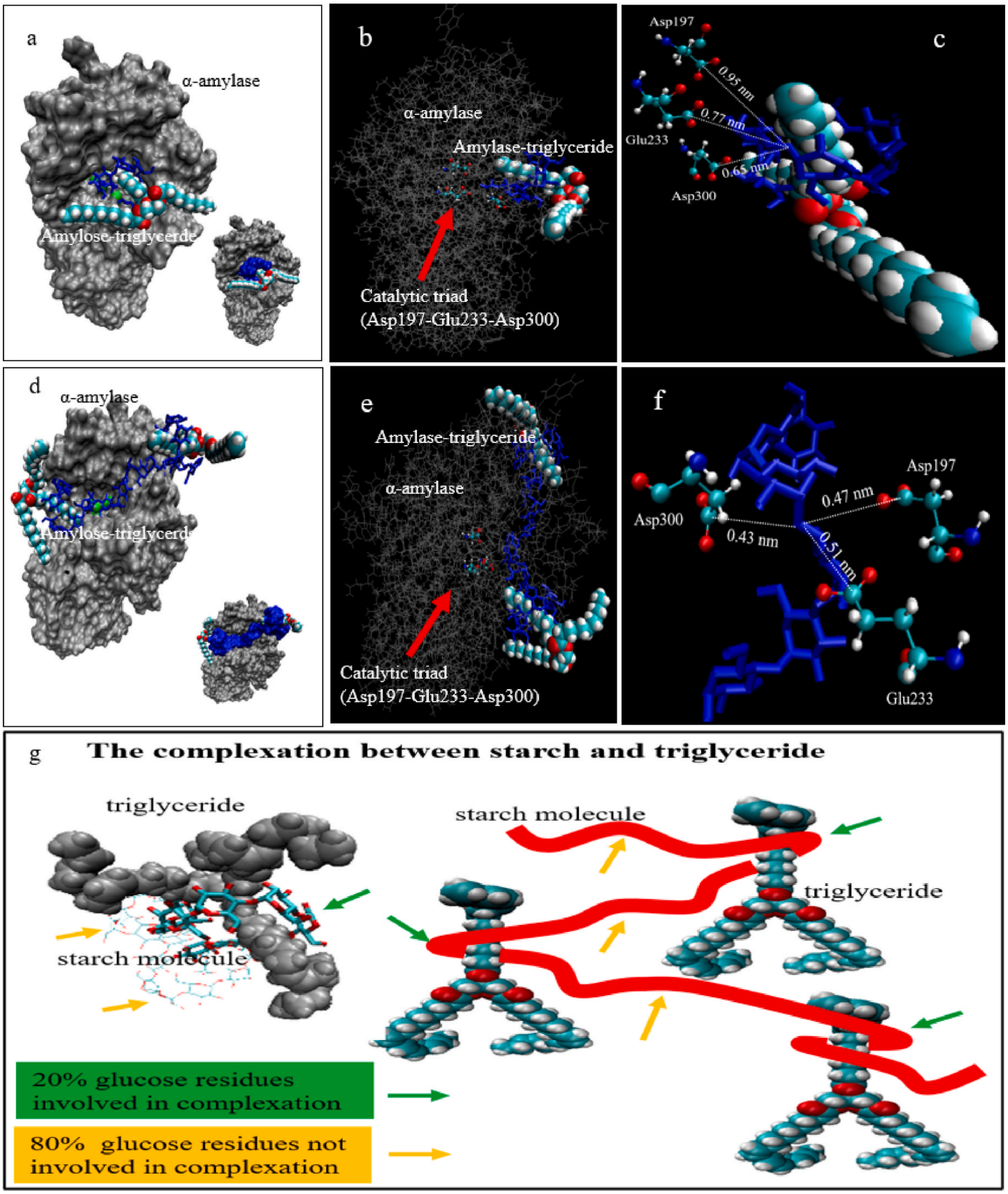

Fig. 3. Interactions between starch-lipid complexes and α-amylase.

small peptide and starch-decanal complexes were designed. Then, the interaction details between α-amylase and the two complexes (starch-small peptide and starch-decanal) were analyzed by using molecular dynamics simulation. It was found that the starch molecules complexed with small peptides or decanal molecules are still not correctly recognized by α-amylase, indicating lipid molecules are not essential for RS5 preparation(Ma et al., 2024). In other studies, researchers have found that adding polyphenols or proteins to starch likewise reduces the rate of starch digestion(Amoako and Awika, 2019; Lin et al., 2020; Gutiérrez and Tovar, 2021).

Therefore, the small molecule compounds with the following characteristics would be ideal raw materials for the preparation of RS5: (1) high complexation efficiency with starch molecules; (2) harmless or beneficial to the human body; (3) easily available and low cost. Small peptides composed of essential amino acids meet all of these requirements. Firstly, the -COOH, -NH_2 or -OH groups in the R group of some amino acids can form hydrogen bonds with starch hydroxyl groups and enhance the interaction of starch molecules with small peptide molecules. Secondly, essential amino acids are important nutrients. Thirdly, small peptides can be artificially engineered and efficiently produced with the help of genetic engineering techniques. In future studies, small peptides may play an important role in RS5 preparation.

3.1.3. Relationship between double-helix structure and RS

Double-helix is the smallest unit of starch crystallisation. The relative crystallinities of different starches generally range from 10 to 45%. It

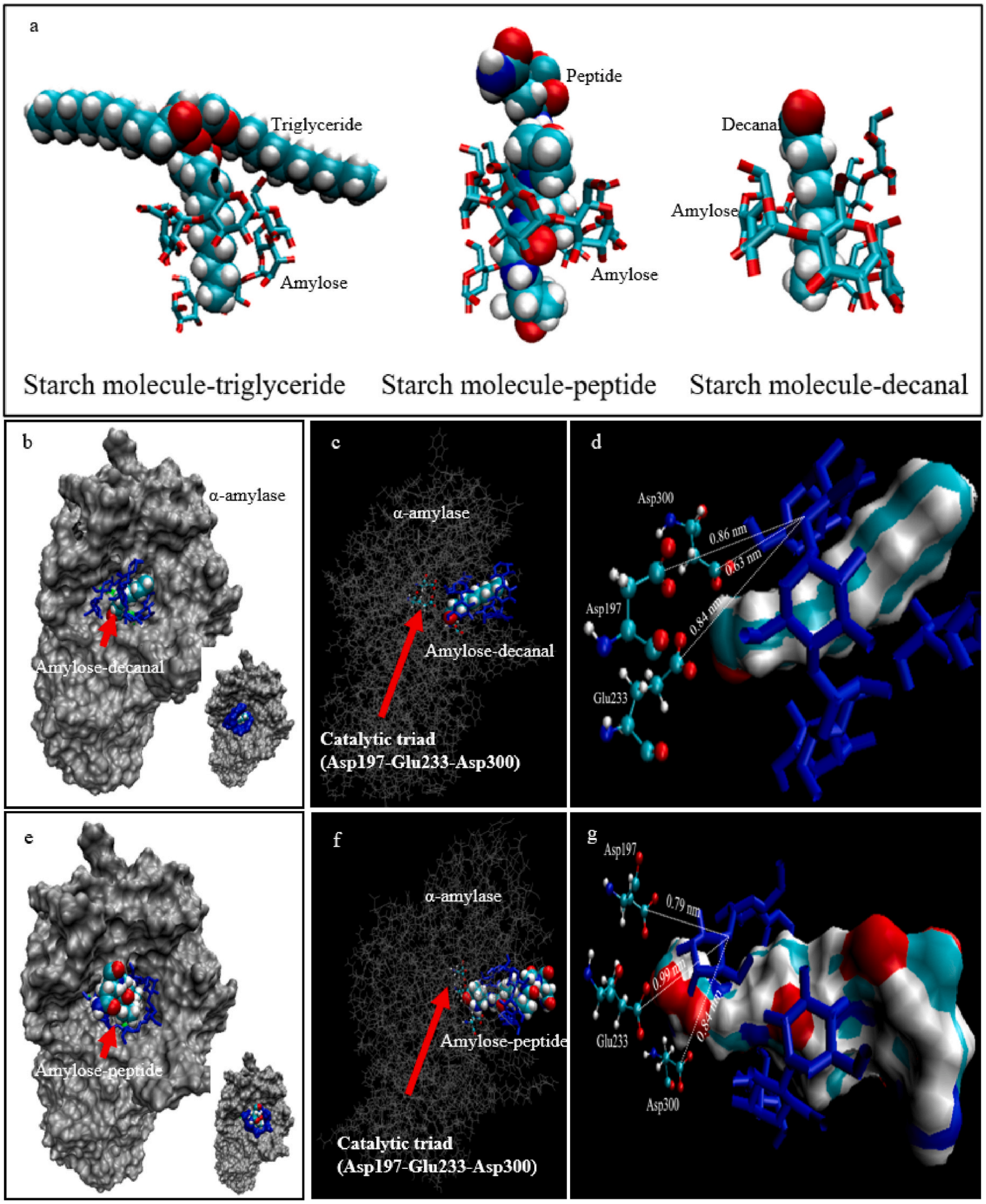

Fig. 4. Interactions between starch-decanal (b, c, d) and starch-peptide (e, f, g) complexes and α-amylase.

can be assumed from common sense that, the crystalline regions will be denser than the amorphous regions, which makes the crystalline regions more difficult to be hydrolyzed by amylases. However, as shown in Table S1, the relationship between the RS content and relative crystallinity of starch is inconsistent between different studies. For example, Hu et al. found that the relative crystallinity and RS content of wheat starch were significantly decreased after high-pressure treatment(Hu et al., 2017). However, Bajaj et al. found that, after high-pressure treatment, the relative crystallinity of wheat starch decreased, but the RS content increased significantly(Bajaj et al., 2021). Xu et al. found that the relative crystallinity of raw potato starch was only 19.6%, but its RS content was as high as 58.1% (Xu et al., 2018). Chi et al. found that raw potato starch had a relative crystallinity of 25.5% but contained only 5.5% RS(Chi et al., 2019).

Current researchers generally agrees that the crystalline structure of starch results is due to the regular arrangement of double-helix structures(Chi et al., 2022; Bertoft, 2017). This double-helix structure is formed by the side chains of amylopectin and is the smallest unit of starch crystallisation(Bertoft, 2017). In addition, it has been shown that in aqueous solution, starch molecules also spontaneously entangle to form a double helix structure(Seung, 2020). In our previous study, the interaction between double-helix starch molecule and human α-amylase was analyzed, to explain the intrinsic relationship between the starch hydrolysis efficiency and crystalline structure at the molecular level (Zhong et al., 2024c).

As shown in Fig. 5, because of the large size of the double-helix molecule, double-helix starch molecule cannot lay flat at the active cleft like the single amylose molecule(Zhong et al., 2024c). During docking, only the head of the double-helix molecule was inserted into the active groove. However, the head of the double-helix molecule could not fully penetrate to the bottom of the active groove and make full contact with the catalytic triad. And the glycosidic bond on the double-helix molecule closest to the catalytic triad is the glycosidic bond between the first and second glucose residues on the A chain of the double-helix molecule. However, the calculated distances of the glycosidic oxygen atoms from the carboxyl groups on Asp197, Glu233, and Asp300 were 1.1 nm, 0.85 nm, and 0. 48 nm, respectively. These distances are not sufficient for the nucleophilic attack of the glycosidic bond by the catalytic triad of α-amylase(Zhong et al., 2024c).

In brief, the double-helix molecule, which is the smallest unit of starch crystallisation, cannot be properly recognized and hydrolyzed by α-amylase. This suggests that, without considering other structural factors, the RS content should be positively correlated with crystallinity. Therefore, in practice, improving the relative crystallinity of starch is an important way to prepare RS.

3.2. Granular lever

It can be observed through an optical microscope or scanning electron microscope that starch naturally exists in the form of granules, with granular sizes ranging from 0.5 to 120 μm. At present, researchers generally agree that the crystallinity, amylose/amylopectin content, and specific surface area of starch granules are all closely related to the RS content(Wang et al., 2023). It has been clarified above that the higher the crystallinity the higher the RS content.

3.2.1. Relationship between amylase penetration and RS

With the help of low-temperature nitrogen adsorption technique, the average specific surface area of starch granules can be analyzed to be around 0.7 m^2/g (Xie et al., 2019; Sujka, 2017). Currently, it is commonly understood by researchers that the larger the specific surface area of a starch granule, the larger its contact area with amylase and the higher the rate of starch hydrolysis(Wang et al., 2023). However, this understanding misses an important point: the specific surface area is tested by nitrogen adsorption. Because the nitrogen molecule is small, it can freely penetrate almost everywhere inside the starch granule. Whereas amylase molecules are too large in size compared to nitrogen molecules (The radius of gyration of α-amylase is about 3.23 nm). Thus, in a real situation, the amylase may not be able to fully penetrate the interior of the granule, and hydrolysis starch granule from both the inside and outside simultaneously. In addition, a study by Chen can be used as a reference. In Chen's study, the starch granules were incubated in fluorescently labelled lipid solutions, to observe the interaction between starch granule and lipid molecules. It was found that only the surface layer of starch granules had a few fluorescently labelled lipid molecules(Chen et al., 2021). And the lipid molecules (It has a length of about 2 nm) were hard to penetrate inside starch granules. Of course, the hydrolysis of starch takes place in an aqueous solution, in which starch granules will slightly swell. However, there are no studies to show whether amylase can penetrate into the interior of starch granules during hydrolysis.

As shown in Fig. S2, by using scanning electron microscope, laser

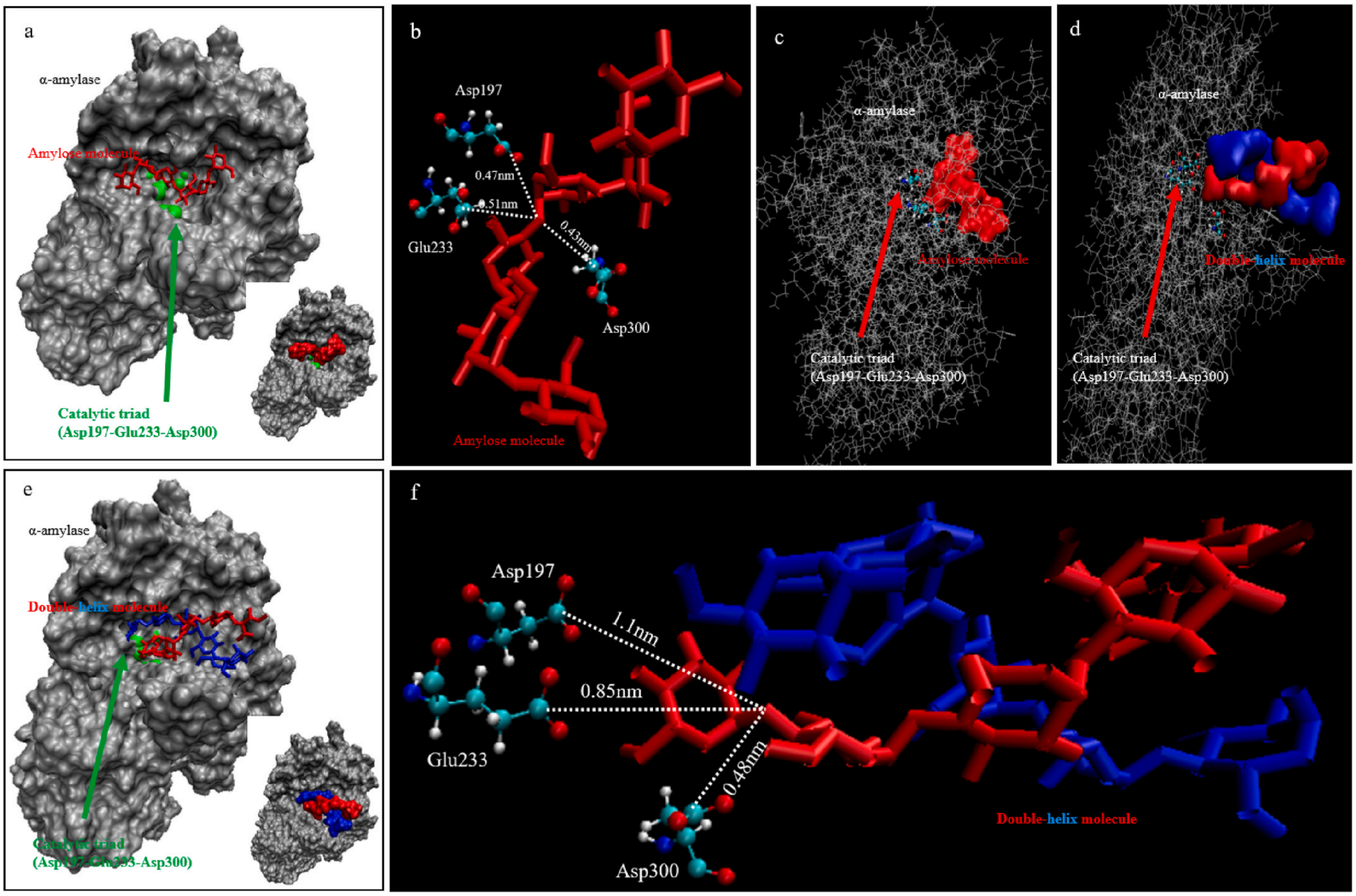

Fig. 5. Interactions between single (a, b, c) and double-helix (d, e, f) starch molecules and α-amylase.

confocal microscope and high-resolution 3D CT, it can be seen that the internal structure of starch granule is hollow(Emma and Alison, 2003; Wang and Copeland, 2015). As shown in Fig. S2d, by fitting the scans of 3D CT, it can be approximately calculated that the volume of the hollow space inside the starch granule is about 30% of the whole granule. If a starch granule could allow amylase to penetrate its hollow parts and allow amylase to hydrolyze it simultaneously from the inside and the outside, then its rate of enzymatic hydrolysis would be greatly increased. In contrast, if the enzyme can only hydrolyze from the surface of the starch granule, then its RS content will rise significantly. By searching the available studies, it can be seen that, the starches from the same species, with the similar crystallinity and similar amylose content, have a significant difference in their RS content. This is most likely due to the difference in amylase penetration(Bajaj et al., 2021; Xu et al., 2018; Chi et al., 2019).

In summary, the specific surface area of the granules is not an accurate indication of the RS content, and a more accurate indication would be the amylase penetration. The key point in preparing resistant starch granules is to inhibit the swelling of starch granules and prevent amylase from penetrating into the interior of the granules. Moreover, in future studies, researchers can try to prepare the fluorescently labelled amylases and analyze the amylase penetration of different starches. If the fluorescent labelling of amylase is difficult, a molecule with a similar size to amylase and can be easily fluorescently labelled can be chosen.

3.2.2. *Relationship between amylose/amylopectin content and RS*

Currently, researchers generally believe that the higher the amylopectin content the higher the hydrolysis rate. This is due to the fact that amylopectin molecules have multiple non-reducing ends, which allows β-amylase to start hydrolysis from multiple non-reducing ends simultaneously(Nakamura and Kainuma, 2022). This is one of the important reasons why amylose/amylopectin content affects RS content. A point that is easily overlooked is that the amylose/amylopectin content also has an effect on the structure of the starch granule, which in turn leads to changes in RS content.

As shown in Fig. S3, a model of the fine structure of starch granule is proposed according to the starch molecular size(Chen et al., 2020c), growth ring structure (Emma and Alison, 2003), and other experimental observations (Wang and Copeland, 2015). Starch granule is composed of multiple growth rings arranged layer by layer. Each growth ring consists of many amylopectin molecules, with a width of about 400 nm. These amylopectin molecules cross-link with each other by forming double-helix structure, making the growth ring structure relatively stable. The longer amylose molecule may traverse and cross-link with multiple growth rings through hydrogen bonding, acting to stabilize the granule structure.

In this starch granule model, the amylopectin molecules are the key for formation of starch granule. It is well known that the amylopectin content of waxy starches can be close to 100%, whereas the amylose content of high-amylose starches can only go up to about 60% or else the granules cannot be formed(Wokadala et al., 2014).

In addition, the amylose molecules may traverse multiple growth rings, randomly distributed throughout the starch granules. It is well known that during starch gelatinization, the higher the content of amylose, the more difficult it is for starch granules to paste and expand (Sandhu et al., 2008) (A simple experiment: by using RVA, it can be tested that the gelatinization temperature of waxy corn starch, normal corn starch, and high-amylose corn starch are about 65 °C, 80 °C, and 100 °C, respectively). This suggests that amylose molecules can cross-link with the amylopectin molecules from different growth rings (like a rope binding multiple growth rings), which in turn prevents the granules from expanding. That is, the amount of amylose determines the stability between different growth rings. A higher amylose content can bind neighbouring growth rings more tightly. Thus, a higher amylose content leads to less swelling of the starch granule, which in turn reduces amylase penetration and increases the RS content. In summary, the higher the amylose content, the higher the RS content. And this is a double effect.

4. Classification of RS

At present, RS is categorized into five types: RS-1 (physically inaccessible starch), RS-2 (resistant granules), RS-3 (retrograded starch), RS-4 (physically or chemically modified starch) and RS-5 (starch-lipid complexes). RS1 is hindered by the food or biological matrix(such as cell wall), resulting in the amylase not being able to reach the starch granules. RS3, RS4 and RS5 are classified from preparation methods. However, they have some overlap. For example, after moist heat treatment, the significant rise in RS is closely related to the rise in crystallinity after starch regeneration. And the RS thus obtained i.e. belongs to RS3 as well as RS4(Van-Hung et al., 2020). In addition, RS5 was once defined as starch-lipid complexes. However, it has been mentioned by researchers that the rate of digestion is also reduced when starch molecules are complexed with other small molecules(Lin et al., 2020). And this has also been confirmed by our previous research(Ma et al., 2024).

With more in-depth studies in the preparation and formation reason of RS, the current classification of RS may not satisfy our current understanding of RS. In this paper, we suggest reclassifying RS from two aspects (preparation methods and formation reasons). As shown in Fig. 6, RS can be classified into four categories in terms of preparation methods: RSn (natural resistant starch), RSb (the RS prepared by biological methods), RSc (the RS prepared by chemical methods, such as the substitution of hydroxyl groups, and the introduction of new groups), RSp (the RS prepared by physical methods, such as annealing, dry-heating and high-pressure treatments). RS can be classified into six categories in terms of formation reasons (the formation reason can be subdivided into two categories: the granular level and the molecular level): RSg-1(starch granules with high relative crystallinity), RSg-2 (starch granules with high amylose content), RSg-3(starch granules with lower amylase penetration), RSm-1(starch molecules with a special conformation that cannot be recognized by amylase, such as the straight molecular conformation formed after heat treatment), RSm-2(starch molecules that form double helix structure), RSm-3(starch molecules that form complexes with other small molecule compounds). The new classification method has some overlap with traditional classification method, but it will be more systematic and clearer than traditional classification method. Taking RS2 in the traditional classification as an example, in terms of preparation method, it belongs to RSn. In terms of formation reasons, it may belong to RSm-1, RSg-1, RSg-2 or RSg-3. Take the RS3 as an example, in terms of preparation method, it may belong to RSc or RSp. In terms of formation reasons, it may belong to RSm-1, RSm-2, RSg-1.

The previous classification method was limited by the research status at that time, which resulted in some shortcomings (including duplication and missing). In the new classification method, RS is classified into 10 types from two aspects (preparation methods and formation reasons). This new classification method is more systematic and accurate than the existing one, which may be more suitable for current research. And it may make it easier to understand the different RS. Of course, this classification may not be perfect. And we hope this new classification will provide reference for RS research.

5. Conclusion

In this paper, first, the essence of starch hydrolysis and the potential hydrolytic modes of amylase were discussed from the molecular level. Second, the formation mechanisms of RS were systematically discussed from the molecular level (molecular conformation, molecular complexation, and double-helix structure) and granular level (amylase penetration rate, amylose content, and relative crystallinity). Third, a new classification of RS is proposed. RS is classified into 10 types from two aspects (reparation methods and formation reasons). Theoretically,

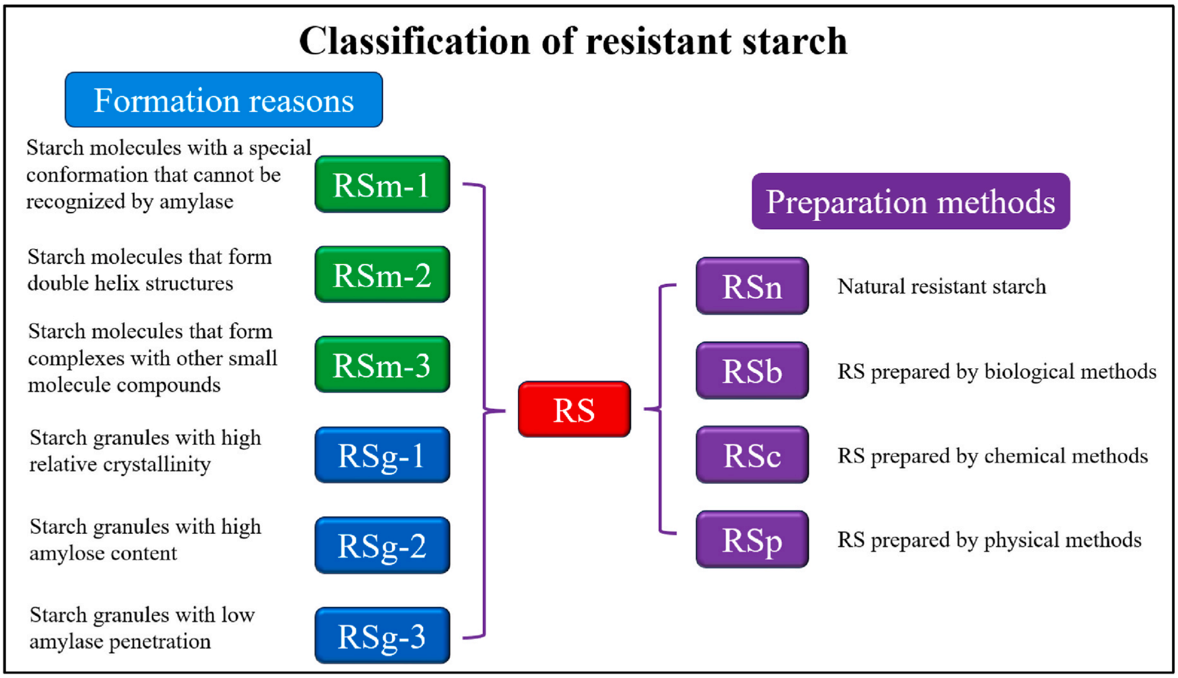

Fig. 6. New classification of resistant starch.

this paper will provide important theoretical support for the research of RS. For practical application, this study can provide an important reference for the preparation of RS.

In this paper, all the analyses of the RS mechanism are only based on the current knowledge of starch structure, which may have certain limitations. For example, the formation of RS may be caused by more than the 6 reasons mentioned in this paper, and there may be other hidden factors that have not been discovered by the researchers. In addition, the new RS classification proposed in this paper may not be perfect either. It needs constant revision and supplementation as the study progresses.

Furthermore, there remain several critical challenges in current research on RS that urgently require resolution. First, the fine structure of starch granule has not been fully elucidated. Clarifying the fine structural details is crucial for understanding the mechanisms underlying RS formation. Second, while many commercially available RS products are obtained through starch retrogradation, this process leads to water exudation and deterioration of elasticity, ultimately compromising product quality and sensory properties. Therefore, improving the textural characteristics of RS-containing products has emerged as a significant research priority. Third, the hydrolytic modes of amylase have not been completely verified. Elucidating the specific mechanisms of amylase-mediated hydrolysis is equally vital for comprehending RS formation, constituting another essential research direction requiring further investigation in RS studies.

CRediT authorship contribution statement

Haixia Zhong: Writing – original draft, Writing – review & editing. **Zhiguang Chen:** Writing – original draft, Writing – review & editing, Project administration. **Yong Han:** Formal analysis, Data curation. **Ke Hu:** Formal analysis, Data curation.

Declaration of competing interest

The authors declare no competing financial interest and no personal conflicts.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2025.101023.

Data availability

No data was used for the research described in the article.

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