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Application of modern instrumental analysis to the determination of starch structure and properties

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Abstract: Starch as a common natural polymer carbohydrate, widely sourced and green renewable, is a cheap biomass material, has been widely used in food processing, pharmaceutical manufacturing and petroleum exploration and other fields.In the process of modernization in China, with the development of China's economy and science and technology, people's demand for functional food, nutritional food and health food is increasing, more and more biomass materials are used in the preparation of functional materials and polymer materials, and the development and application of starch has attracted much attention.In the process of starch research and application, the use of Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and other detection methods, the characterization of starch has become an important means.

1. Application of starch

Starch as a natural biopolymer material has been widely used in food, pharmaceutical, oil drilling and other industries[1,2]. In addition to serving as staple food material in the food industry, starch can also be used as a food additive to regulate the viscosity of food[3,4]. Starch also has important applications in the production of some drugs in the pharmaceutical industry. Adding starch facilitates the fixation of tablets, which plays a fixed and stable role in the drug. In the process of oil drilling, adding modified starch can increase the viscosity of oil drilling fluid, preventing oil drilling fluid from infiltrating the surrounding soil and damaging the soil environment. In the textile industry, the starch slurry film applied to the textile wire can play a lubricating and protective role on the wire.

2. Structure and properties of starch

2.1. Starch structure

The molecular chain of starch is mainly composed of glucose as the smallest molecular unit, which contains amylose and amylopectin. As shown in Figure 1[5], glucose molecules are connected by α-(1→4) bonds to form amylose. Glucose units connected by α-(1→4) bonds and α-(1→6) bonds form amylopectin, with a molecular weight of (10^7-10^9) [5].The structure of starch can be divided into five levels, as shown in Figure 2[6].The first level is amylose chain and amylopectin chain, the second level is amylose molecule and amylopectin molecule, the third level is the double helix structure of starch molecular chain, the fourth level is crystalline layer and non-crystalline layer, and the fifth level is starch particle[6]. Starch has a wide range of sources, mainly from the roots, stems sources, mainly from the roots, stems and seeds of plants. Because of the difference in source plants and plant parts, the diameter of starch particles will be different, and the diameter of different starch particles is between 1-100 μm.

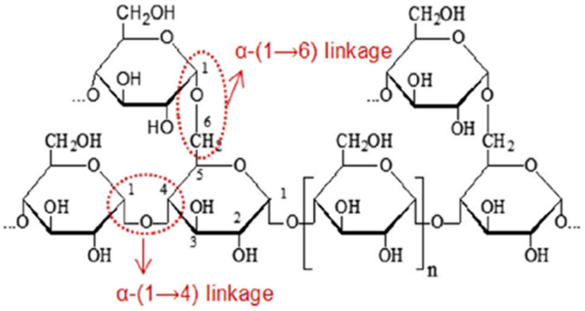

Figure 1: Schematic diagram of the molecular structure of starch [5]

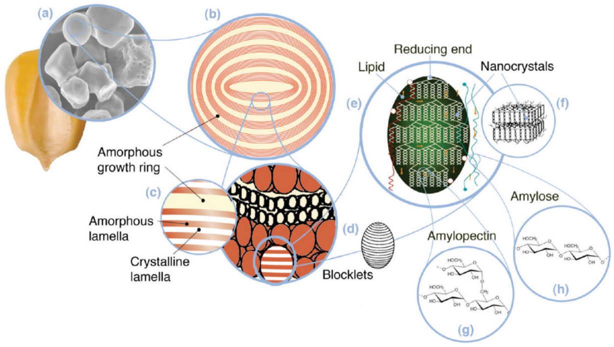

Figure 2: Hierarchical structure of starch [6]

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Starch is a semi-crystalline polymer. Starch can be classified into four different types of crystal structure according to its crystal structure: type A, type B, type C, and type V[7]. Type A starch is compactly arranged with water molecules between the double helices. Type B starch has a longer branched chain than type A starch[8]. Usually cereal starch is type A and tuber starch is type B[7]. Type C starch is an intermediate starch between type A and type B. It can be converted from type A or type B starch under certain conditions, including most legume starches or cereal starches under certain growing conditions. Type V starch is a mixture of amylose and fatty acids and is rarely found in natural starches[9,10].

Amylose is composed of α-(1→6) bonded glucose units. The number of glucose molecules contained in amylose is usually between 60 and 1200. Amylose exhibits a double helix structure and an irregularly coiled structure. The inner part of the double helix is hydrophobic due to hydrogen atoms, while the outer part of the double helix is hydrophilic due to hydroxyl groups[11,12].

Amylopectin is composed of α-(1→6) bond and α-(1→6) bond connected glucose units. Amylopectin contains three types of structure. The first type is a single chain and is connected with the other parts by an α-(1→6) bond. The second type is a starch carrying one or more single chains. The third type is the binding chain of the first two types of amylopectin. One end of the third type of amylopectin is the reduced end of amylopectin[13]. Most of the amylopectin is double helix structure, and part of the double helix structure is involved in the formation of starch crystals[14].

2.2. Starch properties

Natural starch is highly aggregated and has low solubility in cold water. Different types of starch have different solubilities due to the differences in the internal structure of starch, amylose and amylopectin content. Some types of starch can not be dissolved. However, due to the presence of hydrophilic groups in starch molecules, the solubility of starch in aqueous solution gradually increases with the increase of solution temperature[15]. The hydrogen bond between starch molecules can be replaced by water to form paste starch[16], and the amount of short-pectin starch in amylopectin is positively correlated with starch expansion[17].

The digestibility of edible starch can be divided into three types according to the digestibility rate of starch: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS).RDS can be digested rapidly in the human intestine, which is easy to increase the human blood glucose and is not conducive to the control of blood glucose content in diabetic patients.RS is resistant to enzymatic hydrolysis and is not easy to digest in the intestine, which can prevent the risk of diabetes and obesity[18]. The amylose content is an important factor in determining the digestibility of starch. Foods with higher amylose content can effectively control the blood glucose response. The formation of crystals in amylose prevents amylase from approaching the glucoside bonds in the crystallization region, and inhibits the binding sites of amylase active groups from combining with starch molecules, thus making starch resistant to enzymatic hydrolysis[19]. In addition to the amylose content, the crystallinity of starch also affects the digestibility of starch. Starch particles with higher crystallinity have relatively lower digestibility.

Starch dissolved in water after heating, starch particles appear swelling phenomenon, this heat-absorbing expansion phenomenon is called starch gelatinization, pre-gelatinized starch is widely used in food, pharmaceutical industry[20,21]. The crystal structure and double helix structure of starch determine the gelatinization characteristics of starch. The gelatinization characteristics of starch are determined by the crystal structure and double helix structure of starch. The gelatinization characteristics of amylopectin change with the change of molecular weight of glucose molecular chain[22]. In the study of the effect of amylose content on the gelatinization performance of starch, high amylose corn starch shows higher gelatinization temperature than waxy and normal corn starch[23]. But this phenomenon is not caused by the higher amylose content, but because the amylose glucose molecular chain in high amylose corn starch is longer than that in other waxy and normal corn starch, resulting in higher gelatinization temperature of starch. The gelatinization phenomenon is divided into three steps: reversible absorption of water at room temperature, irreversible absorption of water at elevated temperature and decomposition at high temperature.

3. Application of FTIR method to explore starch groups

3.1. The main structure of surface functional groups on starch

FTIR is an important means of characterizing the internal molecular composition and elemental composition of materials, and the use of FTIR can be used for qualitative analysis of the composition of materials. The main measurement is the addition and performance of molecular vibration and rotation. The measurement of FTIR is usually carried out in the mid-infrared region of the material, and the absorption spectrum is between 2 and 25μ. The beam range of the measurement is 4000 cm^{-1} to 400cm^{-1}, and the wave number is 1300 cm^{-1} to 400 cm^{-1} for the fingerprint region, and 4000 cm^{-1} to 1300 cm^{-1} for the characteristic peak region of functional groups. Because the operation of Fourier transform infrared spectrometer is simple and the detection accuracy is high, the use of FTIR to characterize the functional group structure of starch has been widely used.

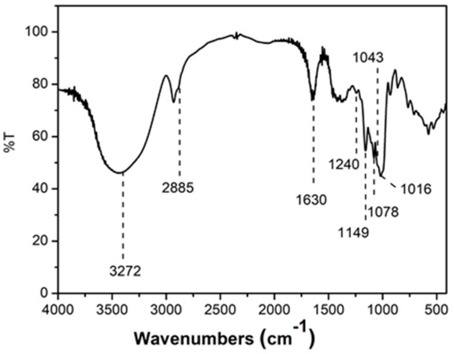

Figure 3: FTIR spectra of cassava starch samples

As shown in Figure 3, the use of FTIR to characterize starch can detect the changes in the structure and structure of starch. People have carried out a lot of research on the characterization of starch and the internal structure changes caused by starch modification by using FTIR technology. The study showed that the use of FTIR to classify different types of starch into A, B and C starch, and the study found that the peak positions at 1157 cm^{-1}, 1105 cm^{-1} and 982 cm^{-1} corresponded to C-O, C-C and C-OH. The three groups in the FTIR on the A starch are greater than the C and B starch samples. The characteristic peak of type A starch appeared at 1022 cm^{-1}, and was significantly higher than that of type B and type C starch at 3600-3000 cm^{-1} and 1200-800 cm^{-1}, indicating that there was stronger hydrogen bond interaction in type A starch, among which the density of hydrogen bond of type B starch was the lowest, but the content of water molecules between double helices of type B starch was higher than that of type A starch[7].

Cael et al. characterized starch by FTIR and pointed out that the absorption band at 1242 cm^{-1} belonged to CH$_2$OH and C-O-H groups, the absorption peak at 1163 cm^{-1} was likely to be the stretching vibration of C-O and C-C, and the absorption peak at 1094 cm^{-1} was likely to be the bending vibration of C-O-H[21]. Kizil et al. characterized starch by FTIR, and measured that the peak at 1630 cm^{-1} was the result of the vibration of soft water molecules absorbed by the noncrystalline area of starch, and the characterization of C-H and o-h could be easily completed by spectral analysis. The FTIR spectrum of starch showed a C-H stretching mode in the range of 2800 ~ 3000 cm^{-1}.Starch showed an O-H stretching mode in the range of 3000 ~ 3600 cm^{-1}. The FTIR characteristic peaks of starch at 900cm-4000cm are shown in Table 1 [24].

Table 1. The main infrared band found in cassava starch samples[24]

Infrared band(cm^{-1})	Vibration mode
3272	O-H stretching (H-bonded)
2885	CH, CH$_2$ stretching
1630	tightly bound water
1336	C-O-H bending,, CH$_2$ twisting
1240	CH$_2$OH (side chain)
1149	C-O-C asymmetric stretching glycosidic bond
1078	C-O, C-C, O-H bond stretching
1043	C-O-H bending (the helices formation)
1016	C-O, C-C stretching，C-O-H bending
993	C-O-H bending
924	C-O-C skeletal mode of α-glycosidic linkage

3.2. Application of FTIR to analysis of group changes of starch before and after modification

The physical and functional properties of natural starch are poor[25], such as insolubility, resistance to enzymatic hydrolysis, poor machinability, poor solubility, poor shear and thermal stability, etc., which can not meet the needs of industrial production. Starch can meet the functional requirements of industrial production after modification, and has a broad market prospect[26]. For example, by modified starch, the solubility of starch is improved, which can better regulate the viscosity of food in the food industry, and as a petroleum drilling fluid, the improvement of starch solubility can improve the water fixation of petroleum drilling fluid and reduce the penetration damage to the surrounding soil. Luis et al. used FTIR to detect the effects of low acetylation and high acetylation on the morphology, physical and chemical properties and structural characteristics of barley starch before and after modification. They found that the absorption peak of low degree of substitution acetylated barley starch and high degree of substitution acetylated barley starch in the range of 900-1250 cm^{-1} corresponded to the C-O stretching vibration, and the peak at 1226 cm^{-1} corresponded to the C-O stretching vibration of acetyl group. The absorption peak at 900-1250 cm^{-1} and 1226 cm^{-1} in the native barley starch sample and low degree of substitution acetylated barley starch was similar, but increased in the high degree of substitution acetylated barley starch, indicating that only part of the acetyl group was introduced into the low degree of substitution acetylated barley starch. When the acetylation reaction occurs in starch molecules, due to the introduction of acetyl group in starch, the peak relative height of O-H stretching vibration at 3000-3900 cm^{-1} and O-H bending vibration at 1650 cm^{-1} decreased, which also confirmed that the barley starch underwent acetylation reaction. At the same time, at 1740 cm^{-1}, the absorption peak of high degree of acetylation of barley starch increased relative to low degree of acetylation of barley starch, which may be due to the vibration of carbonyl in acetylated starch. Thus, low degree of acetylation of barley starch and high degree of acetylation of starch hydroxyl were obtained. The acetylation process reduced the molecular weight of the polymer molecular chain, and the content of short molecular chains in the sample polymer increased, indicating that the starch was depolymerized[27].

4. The appearance of starch was studied by scanning electron microscopy (SEM)

Starch comes from different plants, and its shape and size are different. Common starch includes potato starch, cassava starch, banana starch, mung bean starch, barley starch, etc.Luis et al. acetylated barley starch and compared the surface types of barley starch before and after treatment by SEM, as shown in Figure 4. Figure 4a is natural barley starch, Figure 4b and Figure 4c are low degree of acetylation and high degree of acetylation. When the degree of acetylation is low, the barley starch has a slight change. When the degree of substitution is increased, the surface change of starch is more obvious[27].

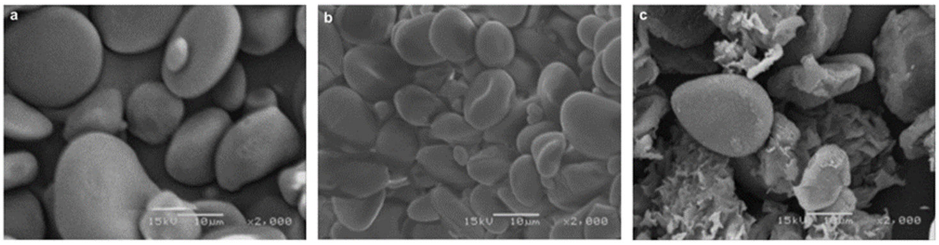

Figure 4: SEM analysis of barley starch before and after acetylation[27]

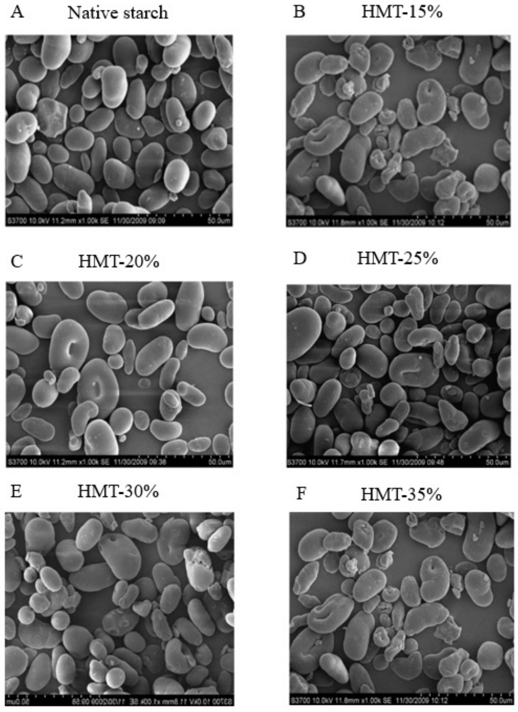

Figure 5: Mung bean starch sample under scanning electron microscope (SEM) at 1000x[28]

Li et al. Heat-moisture treatment (HMT) of mung bean starch, the starch sample was dispersed in an appropriate amount of distilled water, the water was adjusted to 15%, 20%, 25%, 30% and 35% in an airtight container at room temperature for 24 hours, and then heated at 120 °C for 12 hours. Then drying at 45 °C for 12 hours, the surface morphology changes of mungbean starch before and after treatment were analyzed by SEM, as shown in Figure 5. Natural mungbean starch particles were spherical and oval, with different sizes, and smooth surfaces without cracks. After wet-heat treatment, holes or cracks appeared on the surface of starch. Li et al. believed that it may be the recombination of starch chains in particles with weak tissue structure. Therefore, the changes in starch morphology can reflect the interaction of molecular chains in starch particles and the impact of heat-moisture treatment on starch[28].

5. The starch was analyzed by x-ray diffraction (XRD)

XRD is one of the methods for analyzing the crystal structure of substances. Starch, as a polymer compound, has obvious XRD characteristic peaks. Pozo et al. pointed out that the X-ray diffraction patterns of type A, B and C starch were shown in Figure 3[7]. The pattern of type A starch is typical in cereals, and type B starch is typical in tubers and amylose-rich starch. Type C pattern is an intermediate pattern between type A and type B, which is characteristic of most soybean starch and also exists in cereals grown at specific temperatures and hydration conditions. As shown in Figure 6, type A has typical X-ray diffraction characteristics, with the main peaks at 2θ = 15.05°, 17.09°, 17.92° and 23.00%, and the weak diffraction peaks at 2θ = 11.27° and 26.33°; type B at 2θ = 15.26°, 17.21°, 19.75°, 22.32° and 24.08°, and the weak diffraction peaks at 2θ = 5.81° and 26.10°. The characteristic diffraction peaks of type C were observed at 2θ = 15.06°, 17.09°, 19.34° and 22.86°. These results are consistent with previous reports[7].

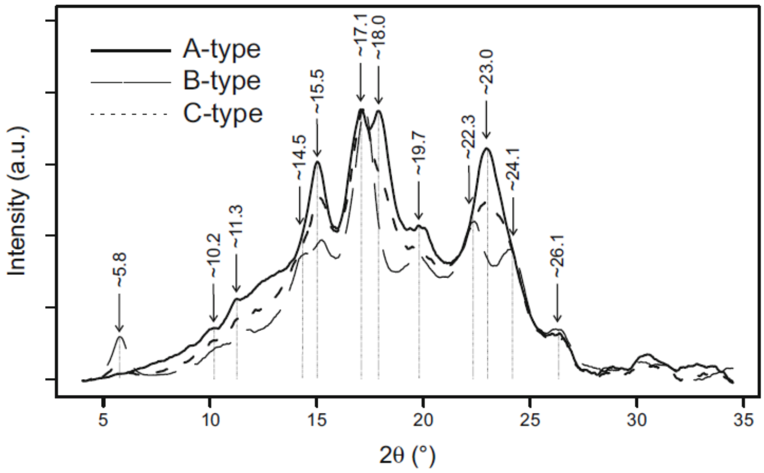

Figure 6: XRD patterns of type A(Mro), B (Pb), and C (Pe) starches [7]

Luo et al. found that the ordinary corn starch and waxy corn starch with a water content of 70% exhibited typical type A X-ray patterns, while the high amylose V corn starch exhibited type B X-ray patterns.Using ultrasound to treat the ordinary corn starch, waxy corn starch and high amylose V corn starch, it was found that the diffraction intensity and diffraction pattern of the treated starch were similar to those of the corresponding natural starch, indicating that the crystal structure of the three corn starches did not change after ultrasound treatment.The crystalline lamellae were composed of amylose double helices, which were stacked in a parallel manner, while the amorphous lamellae were composed of amylose[29].

6. Conclusion

Starch is widely used in industrial and agricultural production, and the function of starch from different plant sources is different. By using FTIR, XRD, SEM and other detection methods, the surface group structure, crystallinity and surface morphology of starch can be studied. At the same time, starch can be modified by chemical and physical means, and the functional changes of starch after modification can be determined by FTIR, XRD, SEM and other detection methods, such as viscosity, molecular weight pH value, gelatinization temperature, etc.

References

1. Ma H , Liu M , Liang Y ,et al. Research progress on properties of pre-gelatinized starch and its application in wheat flour products[J] Grain Oil Science and Technology, 2022, 5(2):87-97.
2. HE H, XIA W, CHEN R, et al. A new pre-gelatinized starch preparing by gelatinization and spray drying of rice starch with hydrocolloids[J]. Carbohydrate Polymers, 2020, 229: 115485.
3. HADIS R, DAGMARA B, ILKEM D, et al. Starch modification through its combination with other molecules: Gums, mucilages, polyphenols and salts[J]. Carbohydrate Polymers, 2023, 314: 120905-120905.
4. MOHAMMED O, BIN X. Review on the physicochemical properties, modifications, and applications of starches and its common modified forms used in noodle products [J]. Food Hydrocolloids, 2021, 112: 106286.
5. Wang K , Henry R J , Gilbert R G .Causal Relations Among Starch Biosynthesis, Structure, and Properties [J].Springer Science Reviews, 2014, 2(1-2):15-33.
6. Le Corre D , Bras J , Dufresne A .Starch nanoparticles: a review. [J]. Biomacromolecules, 2010, 11(5):1139-1153.
7. Pozo C, Rodríguez-Llamazares, Saddys, Bouza R ,et al.Study of the structural order of native starch granules using combined FTIR and XRD analysis[J].Journal of Polymer Research, 2018, 25(12):266.
8. Catherine Gérard, Véronique Planchot, Colonna P ,et al. Relationship between branching density and crystalline structure of A- and B-type maize mutant starches[J].Carbohydrate Research, 2000, 326(2):130-144.
9. Tomy J. Gutiérrez, Tovar J. Update of the concept of type 5 resistant starch (RS5): Self-assembled starch V-type complexes[J].Trends in Food Science & Technology, 2021, 109:711-724.
10. HE H, ZHENG B, WANG H, et al. Insights into the multi-scale structure and in-vitro digestibility changes of rice starch-oleic acid/linoleic acid complex induced by heat-moisture treatment[J]. Food Research International, 2020, 137: 109612.
11. XIANG FJ, SU L, ZHANG XN. Research status of wheat starch [J]. Food Res Dev, 2021, 42(16): 212–219.
12. CHEN JH, WANG YX, LIU J, et al. Preparation, characterization, physicochemical property and potential application of porous starch: A review [J]. Int J Biol Macromol, 2020, 148: 1169–1181.
13. GAO M, JIA JH, ZHANG CJ, et al. Application of extrusion technology in starch and starch-based food

[J]. Sci Technol Food Ind, 2023, 44(14):474–481.

14. CHEN JH, WANG YX, LIU J, et al. Preparation, characterization, physicochemical property and potential application of porous starch: A review [J]. Int J Biol Macromol, 2020, 148: 1169–1181.
15. Yan S L, Chen G Y, Hou Y J, et al. Improved solubility of banana starch by dielectric barrier discharge plasma treatment[J]. International Journal of Food Science and Technology, 2019, 55(2):641-648.
16. JIA RY, CUI CL, GAO L, et al. A review of starch swelling behavior: Its mechanism, determination methods, influencing factors, and influence on food quality [J]. Carbohyd Polym, 2023, 321: 121260.
17. Zhang Z , Li E , Fan X ,et al.The effects of the chain-length distributions of starch molecules on rheological and thermal properties of wheat flour paste[J].Food Hydrocolloids, 2019, 101(4):105563.
18. KUMAR A, SAHOO U, BAISAKHA B, et al. Resistant starch could be decisive in determining the glycemic index of rice cultivars[J]. Journal of Cereal Science, 2018, 79: 348-353.
19. BELLO-PEREZ L A, FLORES-SILVA P C, AGAMA-ACEVEDOA E, et al. Starch digestibility: Past, present, and future[J]. Journal of the Science of Food Agriculture, 2020, 100: 5009-5016.
20. Saravanan D , Muthudoss P , Khullar P ,et al. Pregelatinized Starch: Variability in Gelatinization and its Influence on Product Performance [J]. International Journal of Research in Pharmaceutical Sciences, 2021, 12(1):141-149.
21. Hordiienko O I , Hroshovyi T A .Study of the quantitative factors influence on pharmaco-technological properties of powder masses and tablets with plant extracts and essential oil [J]. Farmatsevtychnyi Zhurnal, 2021(1).
22. Torsten W, James D, Elliot P G, et al. Relations between molecular, crystalline, and lamellar structures of amylopectin[J]. Biomacromolecules, 2012, 13(12):4273-4282.
23. Jane J, Chen Y Y, Lee L F, et al. Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch[J]. Cereal Chemistry, 1999, 76(5):629-637.
24. Zheng M J, You Q X, Lin Y, et al. Effect of guar gum on the physicochemical properties and in vitro digestibility of lotus seed starch[J]. Food Chemistry, 2018, 272: 286-291.
25. Pornpun. L, Jomjai. P, Voranuch. S, et al. The physicochemical properties of a spray dried glutinous rice starch biopolymer[J]. Colloids and Surfaces B: Biointerfaces, 2010, 78: 30–35.
26. SHEN Hs, YU Jt, BAI Jq, et al. A new pre-gelatinized starch preparing by spray drying and electron beam irradiation of oat starch[J]. Food Chem, 2023, 398: 133938-133938..
27. Luis. A. B. P, Edith. A. A, Paul. B. Z. F, et al. Effect of low and high acetylation degree in the morphological, physicochemical and structural characteristics of barley starch[J]. LWT-Food Sci. Technol, 2010, 43:1434–1440.
28. Li S , Ward R , Gao Q .Effect of heat-moisture treatment on the formation and physicochemical properties of resistant starch from mung bean (Phaseolus radiatus) starch[J].Food Hydrocolloids, 2011, 25(7):1702-1709.
29. Luo Z , Fu X , He X ,et al. Effect of Ultrasonic Treatment on the Physicochemical Properties of Maize Starches Differing in Amylose Content [J].Starch - Stärke, 2008, 60(11).