Purification of letinous edodes polysaccharide and its effect on gel properties of rice starch

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Abstract: In this study, lentinan was extracted and purified, and its components were analyzed. The purified letinous edodes polysaccharides were co-pasting with rice starch with different contents (1.0%, 3.0%, 5.0%) respectively. The effects of letinous edodes polysaccharides on the gel properties (transparency, solubility, swelling power, freeze-thaw stability, coagulability and in vitro digestibility) of rice starch were studied. The results showed that after purification, the total sugar content of lentinan reached 55%, protein content was about 8%, ash content was very little, and uronic acid content was as high as 19%. With the increase of lentinan content, the transparency, swelling power and solubility of rice starch decreased significantly, and the freeze-thaw stability and sedimentation was improved, and the swelling power and transparency were directly proportional to temperature. At the same time, lentinan can inhibit the hydrolysis and aging of rice starch to a certain extent, and improve its anti digestion ability.

1. Introduction
Rice is a kind of cereal with a long history of cultivation and economic value in China. Starch is the main component of rice, with a content of up to 90%. It is also an important energy source in the process of human diet[¹]. Rice starch has been widely used in food industry. However, when rice starch is heated, it will produce gelatinization, aging, retrogradation and freeze-thaw stability decline. Jiao Kunpeng's[²] research showed that Water soluble soybean polysaccharide can inhibit the hydrolysis and aging of potato starch, and enhance the anti digestibility of potato starch. Li Yan et al.[³] showed the addition of Auricularia auricula polysaccharides made the corn starch polysaccharide complex system have good stability and anti-aging property, and the effect was more significant with the increase of polysaccharide concentration. Lentinus edodes is a kind of fungus with medicinal and edible value, which has high nutritional, medicinal and health value. The polysaccharide contained in Lentinus edodes is mainly composed of β- D (1 → 3) glucan residue as the main chain and (1 → 6) glucose residue as the side chain[⁴], and contains some natural bioactive substances, which has the functions of antioxidant, anti-tumor and improving immunity. It is speculated that letinous edodes polysaccharides can have some complex interactions with starch molecules. To a certain extent, the physicochemical properties such as rheological properties, thermodynamic properties, gel properties and digestive properties of starch can be changed, which will help improve the processing quality and nutritional quality of starch foods[⁵].
In this paper, letinous edodes was used as raw material to extract polysaccharides, and the effects of different letinous edodes polysaccharides on the gel properties of rice starch were studied. It provided reference for the wide application of polysaccharides and rice starch composite products.

2. Materials and methods

2.1. Materials
Shiitake mushroom was purchased from supermarket of Beijing Agricultural University; monosaccharide standards, including rhamnose, fucose, arabinose, xylose, mannose, glucose and galactose, were purchased from Beijing Changhua Zhicheng Technology Co., Ltd.; acetic acid, acetic anhydride, sodium borohydride, chloroform, trifluoroacetic acid were purchased from China Pharmaceutical Group Co., Ltd.; all other chemical reagents were analytical reagent grade and purchased from Beijing Jinghua Baitai Biotechnology Co., Ltd.

2.2. Experimental methods

2.2.1. Preparation of Lentinan.
The fresh Lentinus edodes were cleaned, sliced, dried at 60 °C for 48 hours, then crushed with a high-speed grinder and passed through a 40 mesh sieve. Refer to Wang to extract polysaccharide[6]. In short, the supernatant was extracted with distilled water (1:20, w / V, g / ml) at 70 ℃ for 1 hour, then centrifuged at 10000 rpm for 10 minutes, and the supernatant was concentrated to 25% of its original volume with a rotary evaporator under vacuum at 60 ℃. Then the supernatant was precipitated and concentrated by adding 4 times volume (volume ratio of concentrated solution to ethanol was 1:4) 80% absolute ethanol. After standing at 4 ℃ for 24 hours, the protein was separated and deproteinized by Sevage method (chloroform butanol, 4:1, V / V). Finally, the polysaccharide was refluxed and dialyzed, and the crude polysaccharide was freeze-dried in vacuum. The lentinan was named SP.

2.2.2. Composition analysis of Lentinan.
The contents of total sugar and protein were determined by phenol sulfuric acid method [7] and Lowry method respectively. The ash content was determined by GB 5009.4-2016 (national standard). The content of uronic acid was determined by carbazole sulfuric acid colorimetry [8].

2.2.3. Determination of transparency.
Refer to the method of Fu Tiantian [9] for measurement. The light transmittance were calculated.

2.2.4. Determination of solubility and swelling power.
Refer to the method of Fu Tiantian[9] for measurement. The solubility and swelling power of rice starch were calculated.

2.2.5. Determination of freeze-thaw stability.
Referring to the method of Xiong Xiaoqing et al[10] and slightly modified. The freeze-thaw stability of rice starch were calculated.

2.2.6. Determination of coagulability.
Refer to the method of Fu Tiantian [9] for measurement. The sedimentation volume ratio of rice starch were calculated.

2.2.7. Determination of in vitro digestibility.
Refer to the in vitro mimic enzymatic hydrolysis method proposed by Englyst[11] with slight modification. The RDS, SDS and RS of each group and the hydrolysis rate in each time period were calculated respectively.
2.2.8. Data processing.
The above experiments were repeated three times, and Data processing and analysis by SPSS 16.0 software, drawing with Excel software.

3. Results and discussion

3.1. Component analysis of polysaccharides

Table 1. Chemical composition of polysaccharides

| Sample | Total sugar (%, w/w) | Protein (%, w/w) | Ash content (%, w/w) | Uronic acid (%, w/w) |
|--------|---------------------|-----------------|----------------------|----------------------|
| SP     | 55.36 ± 0.76        | 8.37 ± 0.23     | 0.43 ± 0.26          | 19.28 ± 0.56         |

The results of the chemical composition of SP are shown in Table 1. The total sugar content is above 55%, indicating that the polysaccharide extracted in this experiment is relatively pure and suitable for structural analysis. After the crude polysaccharide is deproteinized, the protein content is still high, about 8%. The results of Chen et al[12] showed that after deproteinizing by TCA, the protein contained in polysaccharides is still greater than 10%. It showed that the protein in the polysaccharide is not particularly easy to be completely removed, and may have a covalent reaction with the polysaccharide. The ash content of SP is only 0.43 ± 0.26%, indicating that lentinan contains a small amount of minerals. The uronic acid content of SP is relatively high, reaching more than 19%.

3.2. Effect of Lentinan on transparency of rice starch

From Fig 1, with the increase of SP content, the transmittance of rice starch has an obvious downward trend. When the dosage of lentinan is 5.0%, light transmittance decreased to 0.23%. This is consistent with the results of Jiao Kunpeng[2] and others on the effect of water-soluble soybean polysaccharide on the transparency of potato starch paste. Under the effect of temperature, rice starch will completely swell when it absorbs water, and there is no starch granules that are not gelatinized in the process of starch gelatinization. Therefore, it can be inferred that lentinan is likely to be wrapped in the outside of rice starch, which will affect its water absorption expansion in the process of reheating, resulting in incomplete gelatinization and decrease of corresponding transmittance.

![Figure 1. Effect of SP on transparency of rice starch](image-url)
3.3. Effect of Lentinan on solubility and swelling power of rice starch

The swelling power of starch reflects the hydration capacity of starch molecules. The stronger the hydration capacity is, the greater the swelling power is, and the higher the solubility is. It is mainly related to the amylose content and molecular particle size. From Fig 2 and Fig 3, it can be seen that with the increase of SP content, the swelling power and solubility of rice starch decrease significantly, but when the content is 3.0% and 5.0%, the change trend is stable. These results indicated that SP, as a kind of active macromolecule, adheres to the outside of starch. On the one hand, it competes with starch molecules for water, and on the other hand, it hinders the dissolution of amylose and small amylopectin in starch molecules. From the point of view of temperature, the swelling power and solubility of starch increased with the increase of temperature.

3.4. Effect of polysaccharides on freeze-thaw stability of rice starch

Freeze thaw stability is an important index to measure the properties of starch products. According to Fig 4, the higher the content of SP, the lower the water release rate and the stronger freeze-thaw stability. It is consistent with the research of Zhou Zidan[13], Guar gum and sodium alginate can significantly reduce the water release rate of starch gel and improve the freeze thaw stability of starch paste.

3.5. Effect of Lentinan on the coagulability of rice starch

From Fig 5, it can be seen that the coagulability of rice starch is directly proportional to the content of SP, and the change is relatively stable from 1.0% to 5.0%. The reason may be that the action mode of polysaccharide in inhibiting starch sedimentation is mainly with water or starch, reducing the sedimentation of starch molecular chain through hydrogen bonding.
3.6. Effect of Lentinan on digestive characteristics of rice starch

Table 2. Effect of SP on digestive characteristics of rice starch

| Amount of SP added/% | RDS  | SDS  | RS    |
|----------------------|------|------|-------|
| 0                    | 86.69±0.76a | 5.06±0.23a | 3.06±0.13*** |
| 1                    | 72.12±0.45b | 5.68±0.18b | 7.81±0.31** |
| 3                    | 66.91±0.54c | 5.97±0.25c | 10.21±0.30*  |
| 5                    | 64.71±0.37d | 6.28±0.39d | 11.83±0.34*  |

Different shoulder letters in the same column indicate significant differences (P<0.05).

From Table 2, we can see the RDS, SDS, RS values corresponding to different SP added. Compared with the original starch, the RDS value decreased significantly after adding SP. With the increase of the amount of SP, the RDS value decreased steadily, while SDS and RS showed an upward trend. Under the conditions of 3.0% and 5.0% addition, the significant difference decreased. From Fig 6, the starch hydrolysis rate is inversely proportional to the amount of SP. This is consistent with Zhang Can et al[14], who found that after adding inulin, the content of fast digestible starch decreased from 14.51% to 3.06%, and the content of slowly digestible starch and resistant starch increased by 3.67% and 0.20% respectively. It can be concluded that SP can strengthen the anti digestion ability of starch, which is beneficial to human body. The reason may be that SP dissolves in hot water to form colloid, and its viscosity increases, which hinders the action of starch granules and digestive enzymes.

4. Conclusion

The content of total sugar, protein and uronic acid in the purified lentinan was high, but the ash content was low, only 0.43%. Compared with the original rice starch, the addition of SP changed the gel properties of the system. With the increase of SP content, the transparency, swelling power and solubility of rice starch decreased significantly, and the freeze-thaw stability and coagulability were improved. In addition, after adding SP, RDS decreased significantly, while SDS and RS increased in different degrees. When the content is 3.0% and 5.0%, the corresponding value has no significant difference, and the change trend is stable, which indicated that SP can inhibit the hydrolysis and aging of rice starch to a certain extent, and improve the anti digestion ability.

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