Study on Adsorption Conditions of Corn Protein and Starch

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Abstract. This paper mainly measured the adsorption of corn protein on corn starch, studied the changes of protein adsorption under different conditions, and discussed the adsorption mechanism of corn starch and protein. The results showed that when the adsorption time was 1h, the amount of adsorbed protein was the largest. Heating will increase the adsorption capacity; In the range of pH3-9, with the increase of pH value, the adsorption amount of protein firstly increases and then decreases, and the adsorption amount is the largest when pH value is about 6. Adsorption decreases with the increase of ionic strength. The addition of acid pulp can reduce the occurrence of protein adsorption, and the amount of protein adsorption is the least when the acid pulp is added to 50%. Increasing the number of pores and pore size on the surface of starch particles can increase protein adsorption. The adsorption isotherm of corn protein used in this experiment on corn starch granules is S-type, belonging to type II adsorption isotherm.

1. Introduction

Food is a complex system in which many components coexist and interact. Protein, starch and lipid are typical effective components in food. When these typical components coexist, under some suitable physical and chemical conditions (molecular structure, molecular ratio, temperature, pH value, ionic strength, pressure, shear force, etc.), some groups on macromolecules can interact to form complexes, which change the functional properties, processing characteristics and quality of the system (TongFu. kabu, 2007; Liao Ming, 2008).

It was found that corn protein could not be completely removed in the industrial corn starch finished products. SDS-PAGE showed that protein appeared on the surface of starch granules. In the separation process of wet extraction starch, corn starch and corn protein interact with each other, which is considered as an adsorption of rice protein on the surface of starch particles. Some proteins are adsorbed on the surface of starch granules, and the amount of adsorbed proteins varies with the degree of starch refining. Many studies have shown that proteins adsorbed on the surface of starch particles have different degrees of influence on the rheological properties and gelatinization properties of starch, reducing the water absorption and swelling capacity of starch. Eliasson & Tjerneld (1990) studied the adsorption of wheat protein on wheat starch. The results showed that the adsorption of bovine serum protein and low molecular protein on starch granules was very small. In the range of pH 3.1-7.6, the amount of adsorbed protein increased with the increase of pH, and 0.0025MNaCl had the greatest effect on adsorption. Noisuwan (2010) et al. studied the adsorption of milk protein on rice starch and established the adsorption equation, which showed that hydrophobic effect caused protein adsorption on the surface of starch particles. Lu Wei (2012) and others studied the effect of rice gluten on the physicochemical properties of rice starch, and found that adding rice gluten reduced the water holding capacity of rice.
starch, delayed the hydration process, and significantly weakened the gelatinization and gel properties. Therefore, studying the adsorption conditions of starch and protein is of great significance to the separation and purification of starch.

2. Materials and Equipment

2.1. Material
Corn starch, purchased on the market; Corn protein, homemade

2.2. Reagent
95% ethanol, hydrochloric acid, sodium hydroxide, sodium chloride (NaCl), coomassie brilliant blue R-250, phosphoric acid, trichloroacetic acid, bromophenol blue, trihydroxymethyl aminomethane (Tris), glacial acetic acid, glycerol, disodium EDTA, potassium chloride, calcium chloride, ferric sulfate, magnesium chloride, etc. are analytically pure and are purchased from the chemical reagents co., ltd. of the national medicine group. Sour pulp, self-made in laboratory.

2.3. Main Instruments
EL-204 electronic analytical balance Sai Dolis Scientific Instruments Co., Ltd.
DK-S26 Electric Heating Constant Temperature Water Bath Pot Shanghai Jinhong Test Equipment Co., Ltd.
H-16 desktop high-speed centrifuge Changsha Xiangyi Centrifuge Instrument Co., Ltd.
TU-1810 Ultraviolet-Visible Spectrophotometer Beijing shanxi general co., ltd.
HG101-1A Electric Heating Blowing Dryer Nanjing Experimental Instrument Factory
HJ-3 type magnetic stirrer Jintan Chenyang Electronic Instrument Factory
LGJ-25 freeze dryer Beijing Fourth Ring Road Scientific Instrument Factory
PHS-3C digital pH meter Shanghai lida instrument factory

3. Method

3.1. Preparation of corn protein
Soak corn kernels (adding 0.2% sulfurous acid and 0.5% lactic acid) according to wet grinding method, and remove germ after the kernels are soaked and softened. Referring to Li Yan's (2007) method for extracting corn storage protein, the corn grains soaked and germ removed are fully ground in phosphate buffer solution with pH value of 8.5, stirred and extracted in water bath at 4°C for 2h, centrifuged (4000r/min, 20min), supernatant is collected, residue is repeatedly extracted twice, the three supernatants are combined and concentrated, dialyzed and lyophilized to obtain corn protein.

3.2. Determination of protein adsorption rate
500mg of freeze-dried zein is dissolved in 29.7g of water, stirred for 3 hours by a magnetic stirrer to fully hydrate and dissolve the protein, then the solution is centrifuged (1000r/min, 15min) to remove undissolved protein to obtain a completely dissolved zein solution, and the protein concentration is determined by Coomassie brilliant blue method. At room temperature, 5ml of protein solution is added into a test tube containing 1g of starch, and the test tube is placed on a thermostatic vibrator to be slightly vibrated, so that corn starch and protein are adsorbed. After the adsorption is completed, the test tube is centrifuged (1000r/min, 15min) to determine the protein content in the supernatant, i.e. the mass of protein that has not been adsorbed, and the total protein content minus the protein content that has not adsorbed on starch particles is the amount of protein adsorbed on starch particles. The adsorption amount measurement was repeated for three times and averaged. Adsorption rate and adsorption quantity formula:
Adsorption rate of protein on starch (%) = \( \frac{C_0 - C_e}{C_0} \times 100\% \)  

(1)

Adsorption capacity (Q) = \( \frac{(C_0 - C_e) \times V}{m} \)  

(2)

In the formula: \( Q \) represents the amount of adsorption (mg/g); \( C_0 \) represents the concentration of protein solution before adsorption (μg/mL); \( C_e \) is the concentration of protein solution (mg/mL) after adsorption; \( V \) is the volume of adsorbent solution (mL); \( m \) is the mass of starch (g).

3.3. *Determination of protein content*

Coomassie Brilliant Blue Method and Li Zhijiang (2008) Method are adopted. The standard curve equation is \( y = 0.645x - 0.007 \), \( R^2 = 0.9995 \)

3.4. *Determination of adsorption conditions for protein and starch*

The effects of different factors on protein adsorption were investigated. Including adsorption time, protein concentration, heating temperature, pH value of the system, metal ions, ionic strength, acid pulp and starch with different treatments.

3.5. *Handle*

Excel2007 was used for data statistical processing and mapping. SPSS17.0 software was used for average analysis, Duncan multiple comparison was used for difference significance analysis.

4. *Results and Analysis*

4.1. *Effect of adsorption time on adsorption*

The adsorption rate of protein on starch particles was measured in the time range of 10-120min, and the effect of adsorption time on the adsorption rate was investigated, as shown in fig. 1. It can be seen from the figure that the adsorption amount of protein on starch particles increases significantly \( (p < 0.05) \) in 0-60min. when the adsorption time exceeds 60min, the change of protein adsorption rate is not significant, indicating that the adsorption equilibrium is reached after 60min. Therefore, the adsorption time for the following tests was 60min.

![Fig 1. Effect of time on adsorptive ratio](image-url)
4.2. Effect of temperature on adsorption

The adsorption rate of protein on starch was measured in the temperature range of 20℃-90℃, and the influence of different temperatures on adsorption was investigated, as shown in fig. 5-2. It can be seen from the figure that with the increase of temperature, the protein adsorption rate gradually increases. When the temperature exceeds 60℃, the number of adsorbed proteins increases rapidly, and the adsorption rate reaches 59.1% at 70℃, and some gels are found after 30min of centrifugation, which is caused by partial gelatinization of starch. When the temperature exceeds 80℃, the starch granules are gelatinized to a higher degree or even completely gelatinized. After centrifugation, there is almost no supernatant, and protein and starch interact to form gel complexes.

![Fig 2. Effect of temperature on adsorptive ratio](image)

4.3. The influence of pH value on adsorption

Adjust the pH value of the solution to the range of 3.0-9.0 with HCl or NaOH, and investigate the influence of different pH values on adsorption. The results are shown in Figure 3. When the pH value is in the range of 0-6, the amount of adsorbed protein also increases with the increase of pH value. When the pH value of the solution is 6, the adsorption rate reaches 30.2%; When the pH value is in the alkaline range, the adsorption rate starts to decrease with the increase of pH value. This shows that the pH value has a great influence on adsorption. When the pH value of the solution is near the isoelectric point of the protein, the adsorption amount of the protein is the largest, because the solubility of the protein becomes smaller near the isoelectric point, and the force between solute and solvent is relatively weaker, so the adsorption tendency is greater. This result indicates that the isoelectric point of the easily adsorbed corn protein may be around pH 6-7.
4.4. Influence of ionic strength on adsorption

Because Na⁺ has the best inhibitory effect, NaCl is used to study the effect of ionic strength on adsorption. Different concentrations of NaCl were added to the adsorption system to measure the adsorption rate. The measurement results are shown in fig. 5. It can be seen from fig. 5 that with the increase of NaCl concentration, the adsorption amount gradually decreases, indicating that NaCl can destroy the electrostatic attraction between protein and starch particles, making protein unable to adsorb on the surface of starch particles. Moreover, low concentration of salt can improve the solubility of protein. The adsorption amount is closely related to the solubility of solute. Solutes with lower solubility are easier to be adsorbed, so the adsorption amount will be reduced due to the addition of salt (Shen Zhong, 2004). Therefore, as the ionic strength increases, the adsorption rate gradually decreases.
4.5. *Effect of Physalis alkekengi on Adsorption*

Different proportions of acid pulp were added to the reaction system respectively to measure the adsorption rate of protein on starch and to investigate the effect of acid pulp on adsorption. The results are shown in fig. 5. As can be seen from fig. 5, the adsorption rate decreases after adding different proportions of acid pulp. When the amount of acid pulp added is below 50%, the adsorption rate gradually decreases with the increase of the proportion of acid pulp. When the amount of acid pulp added is 50%, the adsorption rate is only 9.3%. The possible reason for this phenomenon is that the acid pulp contains lactic acid, which increases the solubility of protein and thus reduces adsorption. In addition, studies have shown that Lactobacillus paracasei subsp. Paracasei L1 in physalis has a rapid starch sedimentation effect (Zhang Lili, 2010). Because lactobacillus paracasei combines with starch and causes starch to settle rapidly, the adsorption sites and adsorption time of protein and starch are reduced, and starch and protein are separated rapidly, thus causing a decrease in adsorption capacity. With the increase of the proportion of acid pulp, the adsorption rate increases, which may be due to the increase of lactic acid content caused by the excessive addition of acid pulp. High concentration of lactic acid denatures protein and decreases solubility, thus increasing the adsorption amount.

![Fig 5. Effect of Sour liquid proportion on adsorptive ratio](image)

4.6. *Effect of different starches on Adsorption*

The effects of common commercial starch, removed surface binding protein starch and porous starch on adsorption were studied. The experimental results are shown in fig. 6. As can be seen from fig. 6, the adsorption rate of porous starch is the largest, followed by surface-removed binding protein starch. Porous starch is a new type of modified starch. Its surface is full of pores with a diameter of about 1μm, and it penetrates from the surface to the center. This shows that the porous structure of porous starch is an important reason for the increase of adsorption rate. In the study, it was found that after removing surface binding protein with alkaline protease, the number and diameter of micropores on the surface of starch particles increased, which further explained that the micropores on the surface of starch particles had an important effect on protein adsorption. It can be inferred from this that the degree of breakage of starch particles is related to the amount of adsorption. The higher the degree of breakage, the more protein adsorbed. Therefore, in the process of extracting starch and refining starch, the degree of starch damage should be controlled.
5. Discussion

5.1. Solid-liquid interface adsorption
Solid-liquid interface adsorption is a phenomenon of interaction between solid surface particles and liquid molecules, and is one of the most common phenomena in adsorption. For example, adsorption of different proteins on hematite and adsorption of bovine serum proteins on silica, etc. The adsorption of zein on starch particles studied in this experiment can also be regarded as solid-liquid interface adsorption. Compared with solid-gas interface adsorption, the adsorption law of solution is more complex, which is mainly due to the solvent besides solute in the solution. In the adsorption of solid from solution, at least three forces need to be considered, namely, the force between solid and solute on the interface layer, the force between solid and solvent, and the force between solute and solvent in solution. MacRitchie (1978) and Eliasson & Tjerneld (1990) have studied the adsorption of wheat protein on starch granules. It is believed that the increased adsorption amount is related to the molecular weight of wheat protein and the existence of intramolecular and intermolecular disulfide bonds. Noisuwan (2010) et al. studied the adsorption of milk protein components on rice starch, showing that the amount of adsorption of different components is closely related to the rigid or flexible structure of the component protein itself. This indicates that the adsorption of protein on starch particles is related to the properties of protein itself, such as molecular weight, size of molecules in solution, and charge on molecular surface (Horbett & Brash, 1995). It can be seen from SDS-PAGE that the corn protein prepared in this experiment is a mixed protein, but not all proteins are adsorbed on starch particles from the point of view of adsorbed protein types, which indicates that protein adsorption on starch may be selective. In this experiment, the properties of adsorbed proteins, amino acid composition, charge characteristics, protein structure and adsorption capacity of each protein were not studied in depth. Therefore, the adsorption of adsorbed proteins on oil-water interface and solid surface could not be described from the perspective of their own properties, which requires further in-depth study.

5.2. Discussion on adsorption mechanism
In this experiment, the adsorption isotherm of zein on starch granules is Type II. Type II isotherm is of anti-S shape, so it is also called non-S isotherm. In the adsorption process of interaction between solid and liquid molecules, physical adsorption and chemical adsorption can be divided according to the nature of the force. Type II adsorption curve is a typical multi-molecular layer adsorption curve and a common physical adsorption isotherm. According to the multi-molecular layer adsorption theory of BET model, van der Waals gravitation is the main force in physical adsorption. In physical adsorption, there
is not only van der Waals gravitation between adsorbent and adsorbate, but also van der Waals gravitation between adsorbate molecules, so the adsorption layer can make the multi-molecular layer. It can be concluded that the adsorption force of corn protein on starch granules is mainly van der Waals force. In addition to van der Waals attraction, the adsorption behavior of biological macromolecules at the interface is related to electrostatic interaction and hydrophilic and hydrophobic properties of the interface (Elwing et al., 1987). In the results of test 3.4, it is found that the addition of NaCl significantly reduces the adsorption rate. NaCl can affect the electrostatic attraction between macromolecules through ionic strength (Fennema, 1993), which indicates that NaCl reduces the adsorption rate by destroying the electrostatic attraction between protein and starch or protein and protein. thus, it can be inferred that electrostatic attraction is also an important force that leads to the adsorption of corn protein on corn starch.

The premise of BET model multi-molecular layer adsorption theory is to idealize starch particles as solid with uniform surface, but in fact the surface of starch particles is very complex, which shows that the surface characteristics of starch particles also affect the adsorption of zein. Another possible explanation for the increased amount of adsorbed protein is that the protein diffuses into the starch granules. It is reported that there are some pits and holes in the corn starch granules, which are channels connecting the inner cavity of the umbilical part of the starch granules to the outer surface of the starch granules. These holes and channels are large enough to allow water, reagents and enzymes to diffuse into the starch granules. In this case, adsorption on the cavity can occur. In addition, the surface of starch granule also contains starch granule binding protein and fat, which also affect the adsorption of protein (Shewry P R, 2002). Noisuwan(2010)In the adsorption of milk protein on rice starch granules, it was found that the amount of milk protein adsorbed by common rice starch with high binding protein and fat content was higher than that of waxy rice starch with low binding protein and fat content. The results of test 3.6 show that the adsorption amount of protein increases after removing the binding protein on the surface of starch particles with 2%SDS. This indicates that the protein and fat carried by starch particles themselves regulate the adsorption of added protein on starch particles.

Many studies have shown that proteins adsorbed on the surface of starch particles can reduce the water absorption and swelling capacity of starch, and have different effects on the rheological properties and gelatinization properties of starch (Noisuwan, 2010). The study on adsorption in this experiment is not deep enough. It has not studied the microscopic changes of starch particle surface before and after adsorption, the structural changes of protein and starch after adsorption, whether there is specific adsorption, and the nature and structure of adsorbed protein. Therefore, it only indirectly explains why the added adsorbed protein has influence on the gelatinization properties of starch. In other words, in the process of starch absorption, expansion and gelatinization, the adsorbed proteins existing on the surface or pores of starch particles hinder the diffusion of water into the starch particles, thus reducing the gelatinization performance of starch particles.

6. Summary
The adsorption conditions were studied: 1h was the best adsorption time; Heating will increase the adsorption capacity. When the temperature exceeds 80°C, starch and protein are completely mixed together to form gel. In the range of pH3-9, with the increase of pH, the adsorption amount of protein firstly increases and then decreases, and the adsorption amount is the largest at pH 6 or so. The adsorption is also affected by metal ions and ionic strength. The addition of Na⁺ reduces the adsorption rate. In the range of 0-1mol/L NaCl concentration, the protein adsorption decreases with the increase of ionic strength. Fructose and mannose can obviously promote adsorption, while glucose can inhibit protein adsorption. The adsorption of protein can be reduced when the amount of acid slurry is small, but the adsorption of protein increases when the amount of acid slurry is more than 50%. The protein adsorption capacity of different corn starches is different, which indicates that the pores on the surface of starch particles can increase the protein adsorption capacity.
The study on the adsorption isotherm of corn protein on corn starch particles shows that the adsorption isotherm in this experiment is S-type, belongs to type II adsorption isotherm, and conforms to BET model.

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