Research on Improvement Technology of Wet Grinding Process Based on L-Cysteine Fermentation Condition

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Abstract. To study the effects of wet milling, farm Corn; starch; gel filtration chromatography; wet milling; fermentation modification; extrusion enation modification of lactic acid bacteria and extrusion technology on the composition of starch in corn flour. Common corn varieties as a material, corn flour is prepared by wet milling, fermentation modification of lactic acid bacteria and extrusion puffing technology, and starch is extracted from corn flour, using Sepharose CL-2B, Agarose gel column chromatography was used to study the composition of starch. The GPC chart of wet-milled corn flour starch has a bimodal distribution, and the starch molecules are divided into macromolecules Branched-chain region (region I) and straight-branched mixed region (region II), the elution volume of region I is 21-42 ml, and the average molecular weight polymerization degree is 26 632-18 666,The elution volume of zone II is 42-70 ml, and the relative molecular weight average polymerization degree is 18 666-8 046; the starch GPC spectrum of fermented modified corn flour is also double peak Distribution, but the average molecular degree of polymerization of the two regions narrowed, and the starch composition changed; the GPC spectrum of extruded corn flour starch showed a single peak distribution, and the relative The average polymerization degree of the molecular weight is 27 960-9 374. [Conclusion] Starch molecules are degraded during fermentation and extrusion of lactic acid bacteria, and degradation occurs during fermentation The starch molecules are mainly the starch molecules between zone I and zone II, and the high temperature and high shear in the extrusion process make most of the starch molecules in zone I occur degradation.

Keywords: Smart electric energy meter, RFID, data acquisition.

1. Introduction
Starch production has a long history, dating back more than 2000 years. Corn is an important raw material for making starch. 80-90% of the world's starch is processed from corn. The corn wet milling process started from the beginning of the 19th century and has been used continuously through continuous improvement. Before the reform and opening up, my country's starch production was mostly workshop-style production, with a small production scale and low yield. It was not until the early days of reform and opening up that my country introduced advanced production techniques from abroad. In addition to food, corn starch has a wide range of industrial uses, mainly including sugar, paper, packaging, and textile, medical and so on. With the increasing demand for starch products in society,
the continuous advancement of starch deep processing technology and the continuous improvement of the comprehensive utilization rate of by-products, the starch industry has developed rapidly, and the scale has changed to large-scale. My country’s corn starch production bases are mainly concentrated in Shandong, Jilin, and Hebei, and its production accounts for more than two-thirds of the national market. In the past ten years, my country’s starch output has increased year by year. In 2000, my country’s starch output was 5 million tons, in 2005 it was 11 million tons, and in 2015 it was 18 million tons. It can be seen that in recent years it has been a period of increasing domestic corn starch production, with the highest year increasing by 36%. With the improvement of production technology, the total material yield of corn starch in my country has also increased year by year. In 2010, the total dry matter yield of corn starch was 87.23-99.21%, and the starch yield was 64.14-71.2. However, the rapid development of corn starch production in my country also has some problems, such as large energy consumption, large waste water discharge, and serious sulphur pollution. These problems severely restrict the further development of my country’s starch industry. Therefore, corn starch production should develop towards environmental protection and economy, ensure clean production, and promote energy conservation and emission reduction.

Currently, 80%-90% of the starch on the market is corn starch produced by the wet milling process. Corn kernels are immersed in an aqueous solution of sulphurous acid containing 0.2% SO2 at about 50 ℃ for 40-60 h, and corn starch is obtained through processes such as coarse crushing, fine grinding, and gluten separation. During the soaking process, sulphurous acid can prevent the growth of spoilage microorganisms, and SO2 as a reducing agent can reduce the disulphide bonds of the protein wrapped in the outer layer of the corn starch granules, destroy the protein network structure, and free the corn starch. The SO2 used in the traditional wet milling process has a strong corrosive effect on equipment and pipelines. The volatile SO2 in the production process causes serious atmospheric pollution, which is one of the reasons for the haze weather. In order to improve the environmental pollution of the corn wet milling process and find an environmentally friendly corn starch production method, this experiment used L-cysteine as a reducing agent and fermented acid pulp to synergistically separate corn starch. Sour syrup is a pale milky white liquid with a slightly sour taste that is naturally formed in traditional sweet potato starch production, and has the ability to coagulate starch. Adding physisalis in the process of producing starch not only prevents the growth and reproduction of spoilage microorganisms, but also accelerates the separation of starch and gluten. L-cysteine is an amino acid with a reducing sulphydryl group (-SH). It is stable in an acidic environment, and is easily oxidized to generate cystine under neutral and alkaline conditions. Using L-cysteine as a new type of reducing agent to replace SO2 in the corn wet milling process can not only reduce the intermolecular and intramolecular disulphide bonds of corn protein, release corn starch, but also reduce the environment caused by SO2 pollution problem. Physalis not only facilitates the rapid separation of starch and protein, but also helps to improve the properties of corn starch, making corn starch more suitable for the production of vermicelli.

At present, there have been some studies to isolate lactic acid bacteria with flocculation activity in physalis, and the characteristics of physisalis flocculation of mung bean or sweet potato starch have been studied, but there are few related studies on the characteristics of physalis flocculation of corn starch. In the industrial production process of corn starch, lactic acid bacteria are often inserted manually to increase the soaking speed of corn kernels and accelerate the dissolution of protein. Using lactic acid bacteria with flocculation activity instead of ordinary lactic acid bacteria to make acid pulp is used in the production process of corn starch, which can shorten the centrifugation time or replace the centrifugal method to separate corn starch, effectively improve the production efficiency of corn starch and improve the quality of corn starch. In this study, one strain of Lactobacillus piracies L. piracies sub-species L1 with flocculating activity on starch granules isolated from naturally fermented sweet potato physalis was cultured in corn syrup, fermented for a certain period of time to make corn physalis. The effect of the flocculation activity of corn syrup is expected to provide a theoretical basis for the application of corn syrup with high flocculation activity in the corn starch production process [1].
2. Materials and methods

2.1. Materials and reagents
Corn: starch mass fraction 71.26% (dry basis), moisture mass fraction 11.94%; Lactobacillus piracies subspecies L1: extracted from physalis used to produce sweet potato starch, kept in our laboratory. Sodium hydroxide, hydrochloric acid, Coomassie Brilliant Blue G-250, ethanol, phosphoric acid, bovine serum albumin, and L-cysteine are all analytically pure. MRS medium formula: peptone 10.0 g, beef extract 10.0 g, diammonium hydrogen citrate 2.0 g, sodium acetate 5.0 g, dipotassium hydrogen phosphate 2.0 g, manganese sulphate 0.25 g, magnesium sulphate 0.58 g, glucose 20.0 g, Tween 1.0 mL, water 1 000 mL, pH value 6.8.

2.2. Instruments and equipment
WFJ7200 spectrophotometer Unocal; HJ-3 temperature-adjustable magnetic stirrer; pHS-3C pH meter; DHG constant temperature blast drying oven.

2.3. Method
2.3.1. L. piracies L. piracies subspecies L1 cultivation method. Lactobacillus piracies subspecies L1 stored on the solid slope of MRS was inoculated in sterile MRS liquid medium and cultured at 30 °C for 48 h.

2.3.2. Production of naturally fermented corn physalis. Corn kernels were immersed in water for 12 h, then ground (1:3, m/V), and filtered through a 200-mesh sieve. After the filtrate was boiled, it was cooled, and the fermentation broth of L. piracies subcase L. piracies activated by 10% MRS medium was connected to make the corn syrup acidic. The protein in the corn syrup was removed and allowed to stand for 12 h, and the supernatant was taken. Adjust the pH to about 6.8 to make corn juice for physalis. Corn juice was fed with 10% MRS medium activated Lactobacillus piracies fermentation broth and cultured at 30 °C for 48 h to make corn physalis. After every 2 days, pour out 2/3 volume of corn syrup, mix 1/3 volume of corn juice with corn juice and waste corn syrup after starch extraction, and repeat fermentation and cultivation.

2.3.3. L-cysteine and physalis synergistically separate corn starch (LFS). 50g corn kernels are broken into 6-8 petals → add physalis (1:3, m/V) → add 1% volume of physalis to L-cysteine → soak at 30 °C for 48 h → stir well, remove floating Embryo on top of liquid surface → add 1000 mL of water milling slurry → 200 mesh filtration → filtrate is stirred at 30 °C for 1 h → stand still → remove upper layer slurry and gluten → add water to wash → collect starch → dry → after fine grinding pass 200 mesh screen.

2.3.4. SO2 and physalis synergistically separate corn starch (SFS). The corn starch was extracted with 0.2 g/100 mL of sulphurous acid instead of L-cysteine, and the other steps were unchanged.

2.3.5. Infrared spectroscopy. Weigh a small amount of sample, put it in an agate mortar and grind it, then put potassium bromide and continue to grind until it is evenly mixed. After grinding the milled mixture powder, the infrared spectrum was scanned.

2.3.6. Determination of settling properties and settling volume of starch paste. Take 1 g starch sample and add 100 mL of distilled water to prepare starch milk, paste in boiling water bath for 20 min, cool to room temperature, pour starch milk into 100 mL measuring cylinder with stopper In, place the supernatant volume at room temperature for 2, 4, 6, 12, 24, 36 h, respectively. The volume of sinking after 24 h is the settling volume of starch paste.
2.3.7. Determination of freeze-thaw stability of starch paste. Mix starch sample into 6 g/100 mL of starch milk, take 50 mL into a plastic centrifuge tube, heat and paste in a boiling water bath, after cooling to room temperature, add the lid was placed in a -18°C refrigerator to cool, and after 24 h, it was taken out and naturally thawed at room temperature, then centrifuged at 3000 r/min for 20 min, repeatedly frozen and thawed 5 times, the volume of the precipitated water was recorded, and the rate of dewatering was calculated. The formula of water analysis rate is as follows:

\[
P / \% = \frac{m}{M (1-C) \times 71.26} \times 100
\]

2.3.8. Starch extraction. Soak the degreased samples of wet-milled corn flour and fermented modified corn flour in 0.2% NaOH solution for 12 h, remove the supernatant, wash the precipitate with water, centrifuge at 4 000 r/min for 15-20 min to remove the yellow material on the surface, repeated several times, the resulting white precipitate is the starch sample, dehydrated and dried, to be tested. The extraction of starch in the extruded corn flour refers to the separation method of amylose and amylopectin. After separation, the two extracts are mixed and to be tested.

2.3.9. Analysis of starch composition. Gel filtration chromatography (GPC) studies the composition of starch. Sepharose CL-2B external water volume measurement and standard curve preparation Using pure potato pullulan = and glucose to determine the empty volume and total volume of the self-made gel column. Sepharose CL-2B agarose gel was processed according to standard methods and packed into columns, then equilibrated with eluent, and then analysed with standard potato amylopectin and glucose to determine the gel column parameters such as Vo and Ve to determine the column Working status and collection interval. The empty volume of the Sepharose CL-2B gel column was measured to be 38.5 ml; the total volume was 150 ml.

The determination of the average molecular polymerization degree is based on the principle of HPSEC (High Performance Molecular Size Exclusion Chromatography), using standard dextran gels with relative molecular weights of 1.5×10^5, 6.7×10^5, 2.0×10^6, 4.0×10^6 D on Sepharose CL-2B gel chromatography filter column analysis, the sample volume is 1 ml (the concentration of the standard is 2 mg·ml^-1), and a standard straight line is drawn with the elution volume and the standard molecular weight. Find the fitting equation:

\[
y = -379.31x + 34598
\]

\[
( R^2 = 0.9919 )
\]

Among them, \( y \): average molecular polymerization degree, \( DPn \) (average molecular weight, D/162); \( x \): elution volume. Take about 50 mg of defatted corn starch sample, add 1 ml of absolute ethanol (soak the sample), 0.5 ml of distilled water, and 2 ml of mol·L^-1 NaOH. Be careful to rinse the residual sample on the bottle wall, shake the resulting solution sufficiently, and then gradually add 0.5-1.0 ml of distilled water. Heat in a boiling water bath for 20-30 min, while shaking continuously to prevent agglomeration. After the sample is completely dissolved, after cooling, neutralize with hydrochloric acid to pH 6.5-7.0, dilute to 5 ml with distilled water, and filter with filter paper (0.45 micro Filter membrane), take the filtrate for GPC analysis [2].

Before loading, start the constant flow pump, open the lower outlet of the gel column, and elute with 50 mmol·L^-1 NaCl eluent (containing 0.02% sodium adsol) to equilibrate the column for 1 h, then close the constant flow pump and the lower At the outlet, open the column plug and use a pipette to remove the excess eluent from the upper part of the gel column until it is about 5 mm away from the column surface. Open the lower outlet so that the upper liquid surface of the eluent flows tangentially to the column surface and close the lower outlet. Gently add 4 ml of sample solution to be tested (do not use force to prevent damage to the gel column). Open the lower outlet until the upper liquid surface of the sample flows freely to be tangent to the column surface. Close the lower outlet, then carefully add 4 ml
of eluent to the column, connect the tubing, adjust the automatic collector, turn on the constant flow pump and the lower outlet, open the automatic collector and start receiving the eluent. The flow rate was controlled at 14 ml·h⁻¹, and the eluent was 50 mmol·L⁻¹ NaCl (containing 0.02% sodium aside). One tube was collected every 15 min. After the automatic collection is completed, the automatic collector is closed, and the collected sample liquid test tube is taken out. Keep the gel column working and allow 3 times the volume of the gel column bed to flow through the column to clean and equilibrate the gel column. At the end of cleaning, turn off the constant flow pump and the lower outlet [3].

2.3.10. Analysis of visible light absorption spectrum of starch-iodine complex. Weigh 0.5 g iodine and 1.0 g potassium iodide, dissolve with a small amount of distilled water and dilute to 25 mL with distilled water. Weigh 0.200 g of starch sample, disperse it with a small volume fraction of 90% dimethyl sulfoxide (DMSO) solution, and after dissolving, dilute to 50 mL with 90% DMSO solution. Take 1 mL of starch solution sample, add 0.2 mL of iodine colour developing solution, and dilute to 100 mL with distilled water; 0.2 mL iodine colouring solution to 100 mL with distilled water, as a blank sample. The processed starch samples were scanned in the visible light band of 420-800 nm to obtain the visible light absorption spectrum of the starch-iodine complex [4].

3. Results and analysis

3.1. GPC chart and molecular weight distribution of wet-milled corn flour starch
In order to obtain a more accurate and typical corn starch GPC map, this paper selects common corn varieties for measurement and comparison. The results are shown in Table 1 and Figure 1.

| Table 1. GPC chart and molecular weight distribution of wet ground starch |
|-----------------------------------------------|
| **Corn varieties** | **Amylose content** | **Fraction I** | **Elution volume** | **Average degree of polymerization** | **Peak** | **Peak total sugar** |
|---------------------|---------------------|---------------|------------------|-------------------------------|--------|---------------------|
| Yoju57              | 9.594434            |               | 17.5-45.5        | 27960-17339                   | 24.5   | 0.6024              |
| Dongdan60           | 9.401536            |               | 21.0-42          | 26632-18666                   | 24.5   | 0.6752              |
| Zhengdan958         | 9.779079            |               | 21.0-42          | 26632-18666                   | 24.5   | 0.702               |
| Liaodan565          | 9.58023             |               | 21.0-42          | 26632-18666                   | 28     | 0.6744              |
| Hu202               | 7.57524             |               | 21.0-35          | 26632-21322                   | 24.5   | 0.7276              |
| **Corn varieties** | **Amylose content** | **Fraction II** | **Elution volume** | **Average degree of polymerization** | **Peak** | **Peak total sugar** |
|---------------------|---------------------|----------------|------------------|-------------------------------|--------|---------------------|
| Yoju57              | 9.594434            |               | 45.5-63          | 17339-10701                   | 52.5   | 0.2274              |
| Dongdan60           | 9.401536            |               | 42.0-73.5        | 18666-6718                    | 52.5   | 0.2558              |
| Zhengdan958         | 9.779079            |               | 42.0-70          | 18666-8046                    | 52.5   | 0.2786              |
| Liaodan565          | 9.58023             |               | 42.0-66.5        | 18666.98-9373                 | 52.5   | 0.2462              |
| Hu202               | 7.57524             |               | 35.0-77          | 21322-5391                    | 49     | 0.1906              |
As can be seen from Table 1 and Figure 1, the starch samples were analysed by SepharoseCL-2B gel column, and the resulting GPC spectrum showed a bimodal distribution, which divided corn starch into macromolecular region (region I) and small molecule region (region II). The first area is a large molecule, the elution volume is usually 21-42 ml, the relative molecular weight average degree of polymerization is 26 632-18 666, which basically represents amylopectin; the second area is a small molecule, the elution volume is usually 42-70 ml, the relative molecular weight average degree of polymerization is 18 666-8 046, which basically represents a mixture of amylose and amylopectin. The elution curves of the five varieties of corn wet milled starch showed basically the same peak shape in areas I and II. The elution volume range and peak total sugar content were slightly different, indicating that there was a certain difference in starch structure and composition [5].

Table 2. Correlation analysis of amylose and molecular weight distribution

| Correlation coefficient | Amylose | Total sugar content in area I | Total sugar content in zone II |
|-------------------------|---------|------------------------------|-------------------------------|
| X1                      | 1       | X2 0.2867                    | X3 0.0453*                   |
| X2                      | -0.5784 | 1                            | 0.8686                       |
| X3                      | 0.8565* | 0.1034                       | 1                             |

It can be seen from Table 2 that amylose has a certain negative correlation with the total sugar content in zone I, but the correlation is not significant; the amylose content has a positive correlation with the total sugar content in zone II, and the correlation coefficient is 0.8565, with a significant level value of 0.0453 (<0.05), reaching a significant correlation. This further illustrates that amylose is included in the area II of the map.

3.2. The effect of physalis ratio on the increase of soluble protein in the soaking solution and the extraction rate of corn starch

It can be seen from Figure 2 that when the volume ratio of physalis to corn kernels is 2:1, the increase in protein is 0.2175 mg/mL. Later, as the amount of physalis increases, the protein increases. When the ratio of acid pulp is 1:5, the soluble protein increases the most. However, if the amount of physalis is increased, the increase in soluble protein decreases. This is mainly due to too little physalis, which is not conducive to protein dissolution. The increase in the amount of physalis increases the water absorption and swelling of corn kernels, thereby weakening the binding between starch and protein and accelerating protein dissolution. When the acidity in the soaking solution increases, the protein solubility decreases [6].
Figure 2. The effect of physalis ratio on the increase of soluble protein in extract and extraction rate of corn starch

After soaking the corn, add water to grind it and let it stand. Under the action of physalis, corn starch quickly settles. After removing the upper gluten, the starch is collected and dried. As can be seen from Figure 1, as the amount of physalis increases, the quality of starch first increases and then decreases. When the ratio of physalis was 1:4, the starch extraction rate was the highest, being 95.45%. As the amount of physalis increases, part of the protein dissolves, which is beneficial to the release of corn starch. However, the amount of physalis increases, the acidity of the solution increases, and the flocculation activity of Lactobacillus piracies weakens, which is not conducive to the separation of starch and protein in physalis. At the same time, the experiment used whole corn soaking, adding water to grind and settling, and the starch and protein were still connected and not separated. This may be due to the large molecule of L-cysteine, which makes it difficult to enter the inside of the corn endosperm during the soaking process to destroy the combination of corn starch and protein, and cannot release corn starch. Therefore, it is advisable to use granular soaking, and the ratio of acid pulp is 1:4.

3.3. The effect of the amount of L-cysteine on the increase of soluble protein in the soaking solution and the extraction rate of corn starch

L-cysteine has been widely used as a food additive in noodle products. In physalis, L-cysteine can destroy the disulphide bond of the outer layer protein of corn starch, expand the protein structure, and improve the solubility of the protein. As can be seen from Figure 3, with the increase in the amount of L-cysteine, the increase in soluble protein gradually increased. When the mass concentration of L-cysteine is greater than 2.0 g/100 mL, the increase of soluble protein increases slowly. Under the action of L-cysteine, corn starch is released, and it quickly settles under the action of Lactobacillus piracies. When the mass concentration of L-cysteine was 0.5 g/100 mL, the extraction rate of starch was 62.32%, and there was less separation of starch and protein. As the amount of L-cysteine increases, the starch extraction rate increases. When the L-cysteine concentration is 1.0 g/100 mL and 2.5 g/100 mL, the starch extraction rates are 89.45% and 92.71, respectively. After 1.0 g/100 mL of L-cysteine, the starch extraction rate increased slowly.
4. Discussion
For the wet milling process, there are certain differences in the composition and structure of starch between ordinary corn varieties, but the peak shape of the GPC spectrum is more consistent and has a bimodal distribution, and the difference in total sugar content in each interval reflects the components in the starch composition. The ratio is different. This is more consistent with foreign research reports. HPSEC was used to study the starch composition of popcorn, and the resulting spectrum also had a bimodal distribution, but the debranched starch showed a 3-peak distribution. The starch composition of common corn hybrids of different varieties is separated by GPC chromatography column, 1-butanol precipitation or 1-butanol and isoamyl alcohol precipitation, the GPC spectrum of starch also shows a bimodal distribution, and amylose and amylpectin there is no obvious difference in the molecular weight distribution of starch. The proportion of branched chain length of starch obtained by the three methods is relatively similar. HPSEC research shows that both soluble starches are produced during the preparation of starch paste by alkali cooking and stone milling, which contains a small amount of natural amylopectin, amylpectin fragments and a small amount of amylose. Amylopectin fragments are produced during stone grinding and increase with the extension of cooking time. The amylopectin fragments are highly related to the viscosity of starch paste, cooking time, and gel texture. Therefore, the GPC spectrum of starch in the wet-milled corn flour detected by this experiment may not be the GPC spectrum of natural starch, and the phenomenon of amylopectin chain breakage may occur during the wet-milling process [7].

5. Conclusion
There is no new group produced in the corn starch, which is separated from L-cysteine and fermented acid pulp, but part of the structure of CO stretching vibration is destroyed. Compared with commercial starch, the content of corn starch amylose separated by physalis and L-cysteine synergistic reduction is reduced. The settlement volume of LFS is small, the stability of freezing and thawing is improved, and the transparency is increased. Texture analysis shows that LFS has lower hardness and higher elasticity.
It can be seen from the experimental results that L-cysteine and fermentation improve the properties of corn starch, making corn starch suitable for use in frozen products, cold drinks and noodle products. The functionality and application of corn starch separated by physalis and L-cysteine synergistic action need further study.

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