

# Research on Microbial Fermentation of Potato Residue for Animal Feed

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The aim of this study was to research on microbial fermentation of potato residue for animal feed. In the fermentation production of single cell protein (SCP) feed, potato starch residue was employed as main material, and bran together with horse bean straw powder were used as supplementary material. The mixed raw material was degraded with cellulose enzyme, and then was scarified with scarifying koji of *Aspergilla Niger* and *Trichoderma koningii* to form a solid degradation mixture. The rate of inoculating amount was 8:1.5:0.5. The quality index of SCP feed including protein, rude starch and sensory characteristics were analyzed. The results showed that the protein content of the fermented product was increased from 4.08% to 16.52%, raised by 12.44%, and the bad smell was also eliminated after fermentation. Multi-strains fermentation is one of the feasible technique approaches to resolve the problem of potato starch residue.

## 1. Introduction

In recent years, the use of biotechnology, especially microbial fermentation technology to develop new feed resources and to produce protein feed has attracted more and more attention (Ramli et al., 2017). Especially in the 21st century, with the use of enzymes, antibiotics, probiotics, amino acids, vitamins and feed proteins and other feed products produced with microorganism, fermentation engineering technology is more widely used in the feed industry. These products can make up for amino acids in conventional feed, but also make nutrient content in other roughage feed ingredients rapidly transformed to achieve the effect of enhancing digestion and absorption.

The potato, originating in South America, has cultivation history of more than 7,000 years and is an important kind of food crop, feed and vegetable. In 2009, the global planting area of potatoes amounted to 18.7 million hm<sup>2</sup> and the total output reached 330 million tons, second only to those of corn, wheat and rice, becoming the world's top fourth food crops (Lund et al., 2004). At present, China has the largest potato production. According to the United Nations Food and Agriculture Organization in 2008, China's potato planting area reached 85 million and the annual output exceeded 70 million tons. With the potato yield increasing year by year, the market of potato production and processing is paid more and more attention. In the process potato starch production, a large number of potato starch residue will be discharged. In general, the production of per ton of starch brings 7x10<sup>4</sup> kg of starch residue or so. In addition to a small number of protein, the dry residue also contains a lot of starch, cellulose, pectin, amino acids and other components that can be used, which, if not to be used, not only pollute the environment, can also cause the waste of resources. With the increasing awareness of environmental protection, how to make rational use of potato residue has become a bottleneck restricting the development of potato starch processing industry. Therefore, the comprehensive development and utilization of potato residue can not only reduce environmental pollution and relieve worries in the potato starch processing industry, but also has good economic and social benefits.

## 2. Main components of potato residue

In recently years, with the development of potato starch processing industry, people have raised great concern about the treatment of potato starchy waste. It is suitable for fermentation as medium. Moreover, it is also can

be used to extract protein for fodder. To combine with other waste water treatment methods for the processed water, we can realize the recycled utilization or discharge of waste water. The improved method successfully alleviated the inhibition to lactic acid-producing *R. oryzae* growth and fermentation. The yield of acid reaches 12.59g/L, and the conversion rate for glucose is 79.9%, which is increased by 21% than the method of addition with CaCO<sub>3</sub>. The total carbohydrate content has been decreased greatly from 19.6% to 3.8% in the course of fermentation. Potato protein was successfully extracted by a serial of methods including PI sedimentation, centrifugalizing, heating and so on. A kind of proteinase inhibitor was further separated and predicated. The inhibition rate to trypsin activity is 76.82%. The COD value of potato starchy waste water has totally dropped 30.2% after the protein extraction and active carbon adsorption process. So the yield of lactic acid has increased by the coupling process, and this has provided new method for acid fermentation. Not only the potato protein was extracted, but the waste water was purified after the PI sedimentation and heating process.

Potato starch residue is a by-product in the production process of potato starch. The main ingredients are water, cell debris and residual starch granules. Its chemical composition includes a small number of protein, a large number of starch, cellulose, hemicellulose, pectin, free amino acids, oligopeptides, peptides and ash, etc. The main components of potato residue are shown in table 1.

*Table 1: Main components of potato residue*

Component	Wet basis% (w/w)	Dry basis% (w/w)
Solid content	13.0	-
Ash	0.5	4.0
Protein	0.95	8.65
Crude fat	1.24	11.31
Neutral detergent fiber	0.43	3.90
Acid detergent fiber	1.51	13.75
Ca	0.006	0.056
P	0.011	0.098
Starch	4.07	37
Cellulose	1.87	17
Pectin	1.87	17

### 3. Characteristics of potato residue

#### 3.1 Fluid property

Fresh potato residue is very high in water content, up to 80~90%, but does not have liquid fluid properties, showing the typical physicochemical properties of colloid. The moisture in the potato dregs is not firmly bound to the fiber and pectin in the cell debris, but is embedded in the rest intact cells and needs to be removed exchanged to the environment by the cell membrane. Therefore, it is very difficult to remove moisture from potato residue, which is expensive and energy consuming. It is reported that this problem can be solved by adding cell wall degrading enzymes, but the potato residue is large in output and this method is not feasible at present in view of economic benefits.

#### 3.2 Microbial characteristics

Mayer and Hillebrandt, through medium optimization, found 28 kinds of bacteria, 4 kinds of molds and 1 kind of yeasts were carried in potato residue, in total of 15 categories and 33 kinds (Lund et al., 2004). Potato residue has high moisture content, many kinds of microorganisms and is not easy to store and transport, easy to corrupt and produce odor, resulting in environmental pollution.

China is one of the world's largest potato-producing countries. Every year China produces a large number of potato pulps as a result of rapid potato starch production. If the potato pulp is not to be sealed timely and reasonably, it would be a waste of resources and pollution to the environment. So it will be a contradiction to the sustainable development of China's economy. Therefore, it is imperative to explore the timely and reasonable approach of dealing the potato pulp. In this experiment, we use potato pulp as fermentable substrate, chose appropriate accessories, add inorganic nitrogen, and systematically study the change of crude protein content after the fermentation of single or multiple bacterial in order to ascertain the best combination, proportion and number of bacteria; by testing we also ascertain the additive amount of inorganic nitrogen, the best moisture content, fermentation temperature, pH, thickness of fermentable substrate and fermentation time; by L16(54) orthogonal test, we ascertain the optimization number of bacteria, fermentation temperature, additive amount of inorganic nitrogen, thickness of fermentable substrate and fermentation time;

finally, we make sure that the feeding experiment of pigs can reach to a good feeding effect or not. The experiment results show that the best combination of bacteria to fermentation the potato pulp is *Aspergillus Niger*, *Candida tropicalis* *Bacillus Subtilis* and the best proportion is 2:2:1, the best number is 1%, the best additive amount of inorganic nitrogen is 2%. When the thickness of fermentable substrate is 30cm, fermentation temperature is 33°C, pH and moisture content is natural, fermentation time is 48h, and the crude protein content after fermentation can reach to the number 13.5%. After the feeding experiment of pigs, the test group is 610.2g, while control group daily gain is 512.6g, so we can make sure that the product can gain weight significantly; the test group's ratio of material to weight is 3.85:1, while the control group is 4.39:1, so we also make sure the product can lower feed conversion ratio; the test group's ratio of input to output is 1:1.45, while the control is 1:1.35, so we also make sure that the product can increase economic efficiency. The experiment process not only solves the problem of agricultural waste and environmental pollution, but also provides the new type of microbial protein feed. Besides, the technique is simple enough to operate, while can lower production costs and improve economic efficiency. Figure 1 shows the chemistry expression of potato residue process.

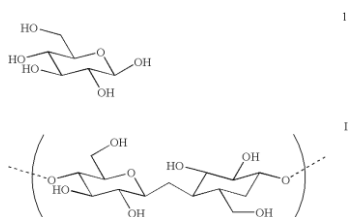


Figure 1: The chemistry expression of potato residue process

#### 4. Potato residue as livestock feed processing and utilization

For the use of potato residue, many domestic and foreign scholars have made various attempts, such as the use of potato residue to extract pectin, dietary fiber, to produce ethanol, enzyme, to prepare novel adsorption materials and to produce protein feed by fermentation.

Table 2: Utilization of potato pulp and residue

Treatment / production methods	Application
Fermentation and multiplication	Animal feed
Enzymolysis	Preparation of pectin or pectin-starch mixtures
Enzymolysis	Converted into sugar and extracting syrup, potato chips and French fries
Hydrolysis	Preparation of alcohol
Liquid-phase extraction	Fertilizer
Diluted with water	Stabilizer (lubricant) in deep well drilling
No treatment	a yeast medium for vitamin B <sub>12</sub> production
No treatment	microbial growth medium for biogas production

At present, there are mainly 3 methods for the development of potato residue, namely, fermentation method, physicochemical method and mixed method. Fermentation method is to use potato residue as culture medium and introduce microorganisms for fermentation so as to prepare various biological agents and organic materials; physicochemical method means that potato residue is treated with physical, chemical or enzymatic methods or extracting effective components from potato residue; the mixed method is a comprehensive method of enzyme treatment and fermentation. Specific research methods are shown in table 2. Domestic research on potato residue is still in its infancy. Hot spots are mainly concentrated in the extraction of active ingredients (such as dietary fiber, pectin, etc.) and the application as a fermentation medium.

$S_{HM}$  solution vectors are randomly generated according to the variable range for each variable. Namely,

$$HMV = \begin{bmatrix} \mathbf{x}^1 & f(\mathbf{x}^1) \\ \mathbf{x}^2 & f(\mathbf{x}^2) \\ \vdots & \vdots \\ \mathbf{x}^{S_{HM}} & f(\mathbf{x}^{S_{HM}}) \end{bmatrix} = \begin{bmatrix} x_1^1 & x_2^1 & \cdots & x_N^1 & f(\mathbf{x}^1) \\ x_1^2 & x_2^2 & \cdots & x_N^2 & f(\mathbf{x}^2) \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ x_1^{S_{HM}} & x_2^{S_{HM}} & \cdots & x_N^{S_{HM}} & f(\mathbf{x}^{S_{HM}}) \end{bmatrix} \quad (1)$$

Hence, we have equation (2):

$$x_i^{new} = \begin{cases} x_i^j, & j \in \{1, 2, \dots, S_{HM}\}, \text{ if } rand < HMCR \\ \bar{x}_i \in X_i, & \text{ otherwise} \end{cases} \quad (2)$$

As for  $x_i^{new}$  from harmony memory, we have equation (3):

$$x_i^{new} = \begin{cases} x_i^{new} + rand * BW, & \text{ if } rand < PAR(\text{Continuous}) \\ (k + \lambda) * x_i^{new}, \lambda \in [-1, 1], & \text{ if } rand < PAR(\text{Discrete}) \\ x_i^{new}, & \text{ otherwise} \end{cases} \quad (3)$$

The worst harmony is replaced with the new harmony, i.e.,

$$\mathbf{x}^{worst} = \mathbf{x}^{new}, \text{ if } f(\mathbf{x}^{new}) < f(\mathbf{x}^{worst}) \quad (4)$$

According to the analysis and description of routing problem in express delivery, the constraints can be expressed as:

(1) Distribution route length does not exceed the maximum value, i.e.,

$$\sum_{k=1}^n d(k-1, k) + d(n, 0) \leq MD \quad (5)$$

(2) The mathematical model of route programming can be defined as

$$L = \min \left( \sum_{k=1}^n d(k-1, k) + d(n, 0) \right) \quad (6)$$

Based on the gradient descent method, node center and base width parameter are:

$$w_j(k) = w_j(k-1) + \eta(y(k) - y_m(k))h_j + \alpha(w_j(k-1) - w_j(k-2)) \quad (7)$$

$$\Delta b_j = (y(k) - y_m(k))w_j h_j \left( \frac{\|X - C_j\|^2}{b_j^3} \right) \quad (8)$$

$$b_j(k) = b_j(k-1) + \eta \Delta b_j + \alpha(b_j(k-1) - b_j(k-2)) \quad (9)$$

$$\Delta c_{j,i} = (y(k) - y_m(k))w_j \frac{x_j - c_{j,i}}{b_j^2} \quad (10)$$

$$c_{ij}(k) = c_{ij}(k-1) + \eta \Delta c_{ij} + \alpha(c_{ij}(k-1) - c_{ij}(k-2)) \quad (11)$$

## 5. Study and application of protein feed from fermented potato residue

### 5.1 Research progress of protein feed from fermented potato residue

Many scholars at home and abroad have done research on protein feed from potato residue. The results showed that the protein content of potato residue could be greatly increased by microbial fermentation, which increased from 4.62% of dry basis before fermentation to 57.49% (Hutvagner et al., 2001). In addition, microbial fermentation can improve the crude fiber structure and bring a faint fragrance to improve palatability. Cellulose and hemicellulose from potato residue were used by Schuction et al. (2004) as fermented materials for the production of microbial protein with the use of *Chaetomium cupreum*. Aziz and Mohsen (Kim, 2005), with potato residue as raw material, produced microbial protein feed by inoculation with *Fusarium* and yeast and treated the fermentation with acid and gamma ray, which greatly improved the yield of protein. After 3-day fermentation, protein yield reached 65.8%. Scientists in Holland (Lee et al., 1993) using by-product of potato starch as raw material and inoculating a kind of special *aspergillus niger*, increased crude protein content in raw materials after fermentation from 3% to 27%. Shi (Lewis et al., 2005) used *Geotrichum candidum*,

*Candida albicans* and *Saccharomyces cerevisiae* for solid-state fermentation of potato residue, which could produce a protein feed with crude protein content of 22.16%. Yang (Yang et al., 2005) used semi-solid method to ferment potato for protein feed. Scholar (Papaioannou et al., 2009) of Taiwan inoculated yeast, mold, actinomycetes on potato residue, added nitrogen source, after solid-state fermentation for 2~5d, produced 15%~32.4% of protein feed. Wang (Ro et al., 2007) first used middle-temperature  $\alpha$ -amylase and Nutrase neutral protease to resolve cellulose and protein in the potato residue and then inoculated protein production strains, *Candida utilize* and *Candida tropicalis*, which could increase protein content in single cell protein to 12.27%.

Although the use of microbial fermentation method can improve the protein content, in order to get a higher protein feed, we need to add a lot of degradable sugar and other nutrients to the potato residue due to low protein content in the potato and high content of crude fiber, less sugar sources for microbial fermentation. This increases the cost of production while increasing the viscosity of potato residue. Therefore, due to the existence of many problems, the production of protein feed with potato residue has not been applied in a large scale.

## 5.2 Application of potato residue-derived protein feed in livestock and poultry

Zhang et al., (2011) added 20% fermented feed from potato residue to the basal ration of white feather broiler as the experimental group and the control group was fed with the basal ration. The results showed that the body weight of the experimental group increased by 29.60%, the fat content of the chicken decreased by 38.47%, the protein content increased by 37.70%, and the liver, kidney and spleen were not damaged (Çalışka, 2014; Jackson et al., 2014). Zhang et al., (2008) used fermented potato starch residue to feed mutton sheep. The experimental group was fed daily with fermented potato starch residue 0.5 kg per sheep. The results showed that compared to the control group, the experimental group increased 17.39% in daily gain and the difference was very significant. The dressing rate was increased by 1.56% and the economic benefit was increased by 30.62%. Zhang et al., (2012) used 70% of the basic diet and 30% of fermented potato residue to feed layers. The results showed that there was no effect on egg performance and egg quality Sezerel and Tonus (2014). Xu et al., (2009) added 20% of fermented potato residue-derived protein feed to the basic diet of rabbits, which resulted in a 20.4% increase in daily gain, a 6.5% decrease in feed conversion ratio and a 0.39% decrease in fat content and had no adverse effects on rabbit immune function.

## 6. Problems in utilization of potato residue

### 6.1 Pretreatment of potato residue

Water content in potato residue is as high as 90% or more. It cannot be used directly as solid medium till water content is decreased to 65%~70%. Potato residue is very sticky, difficult to separate. If simply mechanical method is adopted, water content can only be reduced to about 80% and it is difficult to achieve the required water content of the medium. Studies have shown that chemical reagents can be used to break the water restrained in residual cells of potato residue and then belt filter can be used for mechanical treatment of potato residues so as to solve the problem that it is difficult to filter potato residue. However, the choice of chemical reagents must pay attention to safety and environmental pollution.

### 6.2 Edible safety of potato residue

Potato, an important economic crop for mankind, has a long history of safe consumption. It does not require toxicological tests as feed. However, Potato residue is derived from industrial production and there may be toxin enrichment (such as solanine) and fermented potato residue by different strains contains different ingredients. However, there are few studies on the toxicology of potato residue at home and abroad and there are only experimental studies on the use of part of fermented potato dregs to feed animal feeding. The safety of animal feed is critical to human food safety. Therefore, it is necessary to establish a safety assessment procedure to evaluate the safety of fermented potato dregs as a novel energy feed.

In this experiment, we use potato pulp as fermentable substrate, chose appropriate accessories, add inorganic nitrogen, and systematically study the change of crude protein content after the fermentation of single or multiple bacterial in order to ascertain the best combination , proportion and number of bacteria; by testing we also ascertain the additive amount of inorganic nitrogen, the best moisture content , fermentation temperature, pH, thickness of fermentable substrate and fermentation time; by L16(54) orthogonal test, we ascertain the optimization number of bacteria, fermentation temperature, additive amount of inorganic nitrogen, thickness of fermentable substrate and fermentation time; finally, we make sure that the feeding experiment of pigs can reach to a good feeding effect or not.

## 7. Conclusions

With the potato yield increasing year by year, the market of potato production and processing is paid more and more attention. The aim of this study was to research on microbial fermentation of potato residue for animal feed. In the fermentation production of single cell protein (SCP) feed, potato starch residue was employed as main material, and bran together with horse bean straw powder were used as supplementary material. The mixed raw material was degraded with cellulose enzyme, and then was scarified with scarifying koji of *Aspergilla Niger* and *Trichoderma koningii* to form a solid degradation mixture. In the process potato starch production, a large number of potato starch residue will be discharged. In general, the production of per ton of starch brings 7x10<sup>4</sup> kg of starch residue or so. In addition to a small number of protein, the dry residue also contains a lot of starch, cellulose, pectin, amino acids and other components that can be used, which, if not to be used, not only pollute the environment, can also cause the waste of resources. With the increasing awareness of environmental protection, how to make rational use of potato residue has become a bottleneck restricting the development of potato starch processing industry. The rate of inoculating amount was 8:1.5:0.5. The quality index of SCP feed including protein, crude starch and sensory characteristics were analyzed. The results showed that the protein content of the fermented product was increased from 4.08% to 16.52%, raised by 12.44%, and the bad smell was also eliminated after fermentation. Multi-strains fermentation is one of the feasible technique approaches to resolve the problem of potato starch residue.

## Acknowledgements

This work is supported by the Science and technology department of Hebei province (16226310D-8), Education Department of Hebei Province (ZD2014106) and Animal nutrition and feed science in Hebei Province, the focus of the development of science.

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