Utilization of Potato Protein Recovered from Wastewater of Potato Starch Factory as Cattle Feed*

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Abstract. A large amount of wastewater is discharged from potato starch factories in Hokkaido, Japan. The wastewater contains residual potato constituents such as protein, which are named potato fruit juice (PFJ). A powerful stench is generated from PFJ by anaerobic fermentation. In this study, the isoelectric precipitation technique was applied to recover the potato protein from PFJ. Protein recovery from PFJ by acid isoelectric precipitation at pH 3.0 or less reached 80%. PFJ post-protein recovery at pH 3.0 does not produce a powerful stench. Potato protein recovered from PFJ by acid treatment is a useful feed resource. The PFJ in potato starch factory is a potentially promising resource for the production of potato protein. Wastewater from potato starch factories does not have to be waste or a source of powerful stench, it can be a valuable resource.

Keywords: *isoelectric precipitation; potato starch factory; potato fruit juice; potato protein; feed.*

1 Introduction

Hokkaido has a very large amount of cultivated land (1,153,000 ha), approximately equivalent to a quarter of that of Japan, and develops agriculture that has a high productivity of rice growing, dry field farming and dairy farming [1]. The potato is a staple crop of dry field farming agriculture in Hokkaido, along with wheat, beans and sugar beets. Hokkaido is a major potato farming area; about 80% of all the potatoes grown in Japan are grown in Hokkaido [2]. In particular, Konafubuki, a potato for starch raw materials, occupies about 30% of the potato production in Hokkaido.

Figure 1 shows the block diagram of potato (Konafubuki) starch production and effluent treatment in the potato starch factory of the Japan Agriculture Cooperatives in Koshimizu, Hokkaido. Starch production consists of the five

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processes of conveying, washing, rasping, extraction and starch drying. In the conveying and washing processes, potatoes are transported in water flumes to remove stones, sand, soil, and floating impurities. Then the potatoes are rasped and crude starch milk is extracted by counter-current washing with groundwater. The byproduct of this step is called potato fruit juice, or PFJ. Finally, the starch is dewatered and dried. However, what remains – the PFJ – has a biochemical oxygen demand (BOD) of 40,000 ppm, and includes a large amount of potato protein. PFJ is produced in large quantities and is stored in a reservoir where it is diluted and applied to farmland or subjected to anaerobic treatment and disposed. When PFJ is kept in a reservoir from spring to summer, proteins in the PFJ are metabolized by microorganisms and compounds associated with powerful foul odors, such as butyric acid, valeric acid and isovaleric acid [3]. The residents who live near the potato starch factory are bothered by the powerful stench from the factory [4,5]. Therefore, the recovery of protein from PFJ is required to prevent the powerful stench.

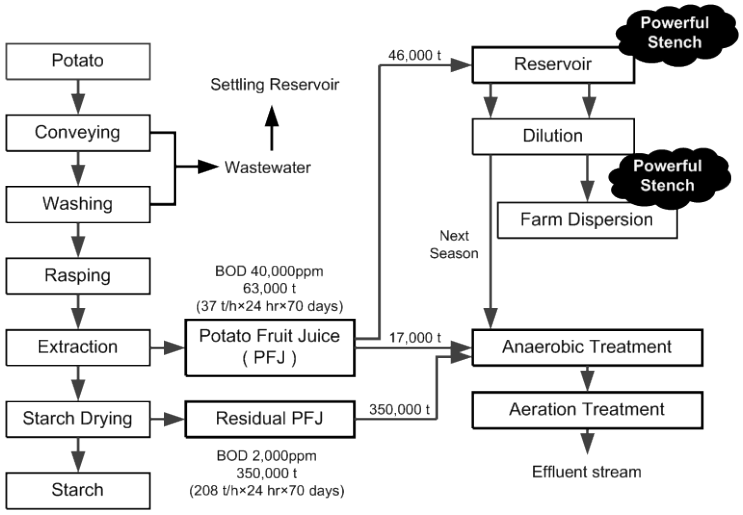


Figure 1 Block diagram of conventional potato (Konafubuki) starch production and effluent treatment in the potato starch factory of Japan Agriculture Cooperatives Koshimizu Town.

Recovery of protein from PFJ with different methods has been reported [6,7]. The most common way to coagulate potato protein from food processing waste effluents consists of pH adjustment, followed by heat treatment at a temperature between 75°C and 120°C [6]. Heat coagulation is another possible process for potato protein recovery. High energy costs induced by the recovery procedures charge the economy of potato starch production [7]. On the other hand, isoelectric precipitation is a method that can simply and efficiently recover

protein from a large quantity of wastewater [8]. In addition, isoelectric precipitation is potentially less damaging to protein quality than heat coagulation. In this study, we have investigated the use of isoelectric precipitation to treat wastewater from a potato starch factory. The aim of this study was to control the powerful stench by recovering potato proteins from PFJ effectively and utilization of utilize the collected protein for cattle feed.

2 Materials and Methods

2.1 Materials

Potato fruit juice (PFJ) was obtained from the potato starch factory of Japan Agriculture Cooperatives Koshimizu Town for four sampling times (11 Oct., 14 Oct., 11 Nov. and 21 Nov. in 2006). 2-D Cleanup kit, ReadyStrip™ IPG Strip – range 3 to 10, RedyPrep 2-D starter kit and Coomassie brilliant blue R-250 for two-dimensional electrophoresis and Bradford protein assay kit were obtained from Bio-Rad Laboratories KK (Tokyo, Japan). Other chemicals used were commercial products of the highest grade available.

2.2 Two-Dimensional Electrophoresis

Isoelectric focusing. A Bio Rad 2-D Cleanup kit, which is based on the protein precipitation by trichloroacetic acid, was used for the preparation of the sample for isoelectric focusing (IEF). IEF was carried out based on the Bio Rad instruction manual using a ReadyStrip IPG Strip, with a pH range of 3 to 10. IEF was carried out using a Protean IEF Cell (Bio-Rad) at 20°C. After passive rehydration for 12 h, the voltage was changed in four steps. First: linearly increasing to 250 V in 15 min, second: linearly increasing to 8000 V in 1 h, third: maintaining at 8000 V until 10000 Vhr, Final: rapidly decreasing to 500V and maintaining for further 24 h.

Second dimension electrophoresis. To equilibrate the IPG strip prior to second dimension electrophoresis, the IPG strip was washed for 20 min in DTT equilibration buffer (RedyPrep 2-D starter kit, Equilibration Buffer I, Bio-Rad), and then in iodoacetamide equilibration buffer (RedyPrep 2-D starter kit, Equilibration Buffer II, Bio-Rad) for a further 10 min. The IPG strip was laid on 12% polyacrylamide gel (8.0 × 7.3 cm) containing SDS and Tris-HCl buffer. SDS polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli [9].

Gel staining. The gel was washed in distilled water and by Coomassie brilliant blue R-250 (Bio-Rad). A two-dimensional electrophoresis gel image was analyzed by Quantity One software (Bio-Rad).

2.3 Effect of pH and Acid Species on Protein Recovery from PFJ (Isoelectric Precipitation)

Effect of pH. 12 M hydrochloric acid was added to 80 ml PFJ in a 100 mL tempered hard-glass vial with screw cap and pH was adjusted to 5.0, 4.0, 3.0 and 2.0. The acidified samples were left to settle for 24 hours at room temperature and then centrifuged until clear supernatants were obtained. The soluble protein concentration in the supernatants was estimated. The experiment as described above was carried out four times.

Effect of acid species. 12 M Hydrochloric acid, 18 M sulfuric acid, 88wt% formic acid, 85-92wt% lactic acid and 99.7wt% acetic acid were added to 50 ml PFJ in a 100-mL tempered hard-glass vial with screw cap and pH was adjusted to 3.0. The vials were allowed to settle for 24 hours at room temperature after the addition of the acid. The sample was then centrifuged until clear supernatants were obtained. The soluble protein concentration in the supernatants was estimated. The experiment as described above was carried out three times.

2.4 Determination of Protein Concentration

Routine determination of soluble protein concentration was estimated by the Bio Rad Protein Assay based on the Bradford dye-binding method [10] with bovine serum albumin (BSA) as standard. A standard curve was prepared by plotting absorbance at 595 nm (SHIMADZU, MultiSpec-1500, Photodiode array spectrophotometer) versus true protein concentration of BSA.

3 Results and Discussions

3.1 Electric Charge of the Potato Protein from PFJ

The PFJ samples contained 95.5wt% water and 4.5wt% solids. The solids were 55% protein, 21% carbohydrate, comprising amino acids, sugars, and organic acids, 2% fat, and 22% ash, including minerals. The main contents of the PFJ were water and protein. The highly concentrated protein of PFJ is converted into powerful, foul odors, such as butyric acid, valeric acid and isovaleric acid that are designated malodorous substances under the System of the Offensive Odor Control Law in Japan [3].

Figure 2 shows the two-dimensional electrophoresis of PFJ with isoelectric focusing, using an IPG strip with a pH range of 3 to 10. The gel was stained with Coomassie brilliant blue; the left-most lane is a molecular weight marker. The PFJ showed a strong band under an isoelectric point of about 5 and the

molecular weight determined by SDS-PAGE was about 40 kDa. Soluble potato proteins are mainly composed of patatin and protease inhibitors [11].

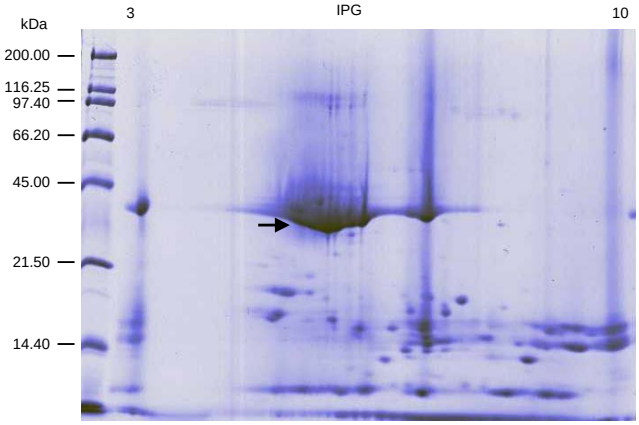


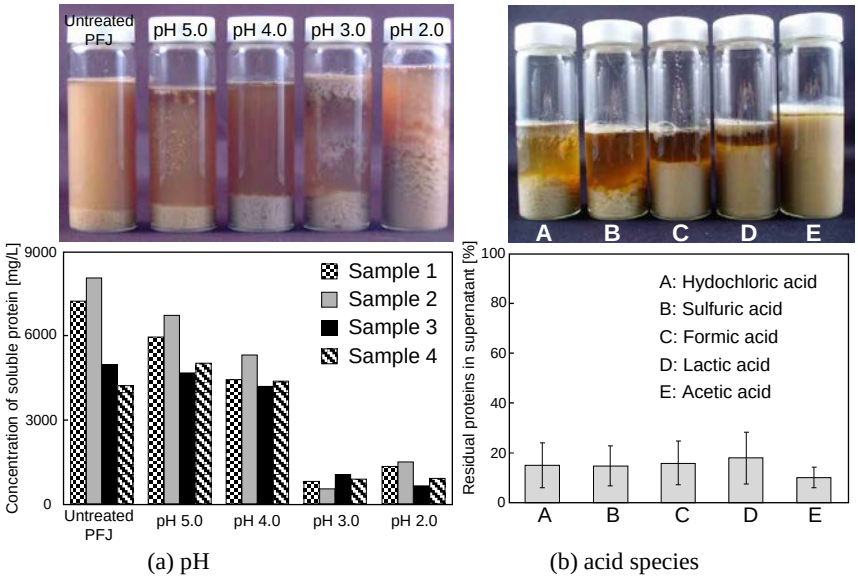
Figure 2 Two-dimensional electrophoresis of PFJ. Potato fruit juice was analyzed by 2D electrophoresis. The pH gradient in the immobilized pH gradient gel strip (ReadyStrip™ IPG Strip, BIO RAD) ranged from with a pH range of 3 to 10, thereby separating the acidic and alkaline proteins. The gel in the SDS dimension was 12.0% acryamide gel. The gel was dyed by Coomassie brilliant blue. The major proteins in PFJ were acidic proteins (shown by arrows).

Protein recovery from industrial PFJ is presently achieved through heat coagulation by steam injection after pH adjustment (i.e. isoelectric precipitation) [7,8]. However, due to the high cost of thermal energy in Japan, heat coagulation is not well suited to this purpose. Based on the isoelectric properties, the electric charge of proteins would become zero at an acidic pH and the solubility of the proteins would decrease, causing the proteins to precipitate without requiring thermal energy. Therefore, isoelectric precipitation as a technique to remove large quantities of protein in PFJ without heat coagulation was investigated.

3.2 Effect of pH on Protein Recovery from PFJ

Protein concentrations of PFJ collected on four sampling days are shown in Figure 3(a) (left side, *untreated PFJ*). The potato starch factory has the capacity to produce starch from potato. Therefore, the potatoes are temporarily stored in farmlands or the factory during the harvesting season. The protein concentrations of PFJ differed considerably by sampling day, which reflects the storage period of the potato.

Hydrochloric acid was added to PFJ to adjust the pH to 5.0, 4.0, 3.0 and 2.0. The concentration of soluble proteins for 24 hours after the addition of the hydrochloric acid is shown in Figure 3(a). At pH 3 or less, the amount of protein remaining in the supernatant decreased considerably, indicating that 80% of soluble proteins could be recovered from PFJ by isoelectric precipitation. Further, the sedimentation at pH 3.0 or 2.0 after 24 hours at room temperature was different, as shown in Figure 3(a) (two glass vials from the right in photograph), the particle sizes of the precipitates were also different.



(a) pH (b) acid species

Figure 3 Effect of pH (a) and acid species (b) on protein recovery from PFJ. (a) Sampling day; Sample 1:11 Oct. 2006, Sample 2: 14 Oct. 2006, Sample 3: 11 Nov. 2006, Sample 4: 21 Nov. 2006, (b) Acid species; A: hydrochloric acid, B: sulfuric acid, C: formic acid, D: lactic acid, E: acetic acid.

Figure 3(b) shows the effect of acid species on protein recovery from PFJ. Hydrochloric acid, sulfuric acid, formic acid, lactic acid and acetic acid were added to PFJ and pH was adjusted to 3.0. For all acid species tested, 80% of soluble protein could be recovered from PFJ. However, the height of the interface between the precipitation layer and the supernatant after setting for 24 hours was different for each acid species. It was revealed that the particularly strong acids, such as hydrochloric acid and sulfuric acid, showed relatively fast sedimentation rates. Sulfuric acid is a commodity in the chemical industry and relatively inexpensive. The technique using sulfuric acid is established safely and surely. Therefore, from the viewpoint of running costs and plant design, it

was decided to use sulfuric acid for isoelectric precipitation to treat wastewater from the potato-processing factory.

3.3 Utilization of Potato Protein Recovered from PFJ

Potato protein recovered from PFJ is a useful resource. Potato protein is of relatively high nutritional quality–comparable to that of whole egg–and therefore it has high potential for use in food products for mammals [11,12]. In 2013, the prices of soybeans, wheat and corn have all soared to 1.7 to 2.6 times their levels in 2006. Over the past four years, the price of the main grains used in cattle feed have all increased. The rising grain prices have resulted in a considerable increase in mixed ration feed prices or mixed feed prices. Farmer requirements for dairy cattle feed are, first, high nutritional value for milk-producing cows, second, stable supply, and third, low cost. Potato starch factories have a considerable potential as a feed ingredient supplier for dairy production. Since it is not influenced by grain market prices, it may be a more stable supply.

Here, we compare potato protein to other agricultural waste products used in cattle feed. Wheat bran is considered to be an excellent source of natural food fiber. Hokkaido is a grain producer with wheat as the main crop. Wheat bran consists of pieces of grain husk separated from flour after milling. Potato pulp is the residue from the rasping step performed by potato starch factories and is an excellent source of dietary fiber. To this line-up we will add potato protein recovered from PFJ. Typically, the cost of 4 kg of mixed feed (corn, wheat and soy bean meal) is $2.67. On the other hand, the nutritional equivalent based on calorie and protein contents derived from agricultural wastes of wheat bran, potato pulp, and potato protein derived from PFJ would cost $0.72. The cost of feed prepared from agricultural wastes is 70% lower than the cost of mixed feed.

4 Conclusion

Figure 4 shows the new treatment process at the potato starch factory managed by the Japan Agriculture Cooperatives in Koshimizu, Hokkaido. Isoelectric precipitation is carried out by the continuous admixture of sulfuric acid to PFJ. Isolation and de-watering of potato protein is performed by a centrifugal separator. Protein recovery from PFJ by acid isoelectric precipitation reaches 80% and the resulting potato protein is a useful cattle feed resource. The supernatant after centrifugal separation is temporarily retained, but there is no longer the problem of the powerful stench. It was regarded that sulfuric acid remains in the supernatant and prevents the proliferation of microbes that metabolize potato protein in PFJ. Finally, the supernatant in the reservoir is

subjected to anaerobic treatment or is neutralized by lime and spread on farmland.

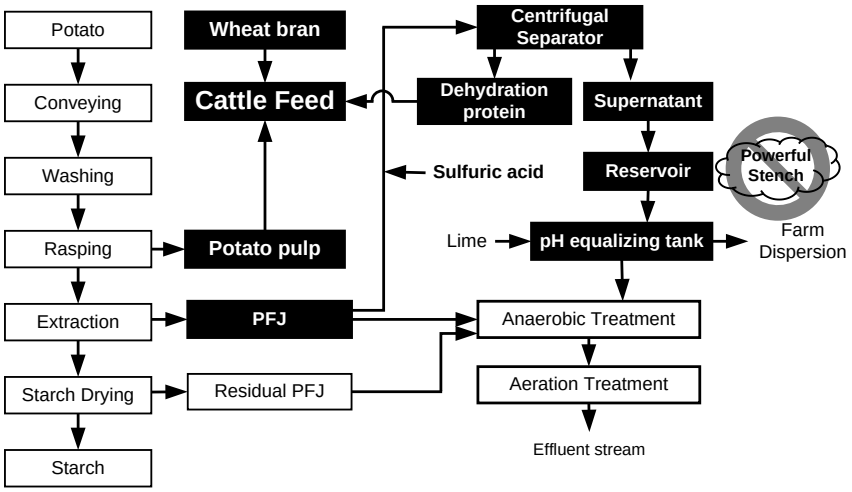


Figure 4 Block diagram of potato (Konafubuki) starch production and new effluent treatment in the potato starch factory of Japan Agriculture Cooperatives Koshimizu Town.

This study had two aims. First, to find a way to control the powerful stench associated with effluents from the potato starch factory, as well as extract the fermentation substrate, potato protein. Second, to use the collected protein effectively. To control the powerful stench and to use the collected protein as cattle feed were simultaneously accomplished in the potato starch factory managed by the Japan Agriculture Cooperatives Koshimizu Town. Through this study, we came to understand that the wastewater from potato starch factories does not have to be waste or a source of powerful stench, but can be a valuable resource.

References

[1] Statistical information (Toukei-jyohou, in Japanese), Ministry of Agriculture, Forestry and Fisheries of Japan, http://www.e-stat.go.jp/SG1/estat/List.do?lid=000001118102 (8 September 2014).

[2] Statistics of Agriculture, Forestry and Fisheries (Nourin-suisan-toukei, in Japanese), Ministry of Agriculture, Forestry and Fisheries of Japan, http://www.maff.go.jp/j/tokei/kouhyou/sakumotu/sakkyou_yasai/pdf/syukaku_barei_13.pdf (8 September 2014).

[3] Pamphlet, Guide of System of the Offensive Odor Control Law (in Japanese), Ministry of the Environment of Japan, http://www.env.go.jp/air/akushu/law_tebiki/03.pdf (14 September 2014).
[4] Research report, starch (in Japanese), August, 2008 issue, Agriculture & Livestock Industries Corporation, http://www.alic.go.jp/starch/japan/report/200808-02.html (14 September 2014).
[5] Mayanagi, M., *Utilization of Wastewater from Potato Starch Factory* (Text in Japanese), Agriculture & Livestock Industries Corporation web, June, 2011 issue, http://www.alic.go.jp/joho-d/joho08_000077.html (14 September 2014).
[6] Knorr D., *Effect of Recovery Methods on Yield, Quality and Functional Properties of Potato Protein Concentrates*, J. Food Sci., **45**, pp. 1183-1186, 1980.
[7] Bergthaller, W., Witt, W. & Goldau H.-P., *Potato Starch Technology*, Starch, **51**, pp.235-242, 1999.
[8] Van Koningsveld, G.A., Gruppen, H., de Jongh, H.H.J., Wijngaards, G., van Boekel, M.A.J.S., Walstra, P. & Voragen, A.G.J., *The Solubility of Potato Proteins from Industrial Potato Fruit Juice as Influenced by PH and Various Additives*, J. Sci. Food Agric., **82**, pp.134-142, 2001.
[9] Laemmli, U.K., *Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4*, Nature, **227**, pp. 680-685, 1970.
[10] Bradford, M.M., *A Rapid and Sensitive Method for The Quantitation of Microgram Quantities of Protein Utilizing The Principle of Protein-Dye Binding*, Anal. Biochem., **72**, pp. 248-254, 1976.
[11] Van Koningsveld, G.A., Gruppen, H., de Jongh, H.H.J., Wijngaards, G., van Boekel, M.A.J.S., Walstra, P. & Voragen, A.G.J., *Effect of pH and Heat Treatment on the Structure and Solubility of Potato Proteins in Differed Preparations*, J. Agric. Food Chem., **49**, pp. 4889-4897, 2001.
[12] Fukushima, K. & Ohba, K., *Kagaku-to-Seibutsu* (in Japanese), **49**, pp. 134-142, 2001.