Food Sci. Technol. Res., **15** (6), 653–658, 2009

Enzymatic Hydrolysis and Ethanol Fermentation of By-Products from Potato Processing Plants

Sunao YAMADA[1,2], Noriyuki SHINOMIYA[3], Kiyoshi OHBA[3], Mitsuo SEKIKAWA[1] and Yuji ODA[4*]

[1] *Graduate School of Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan*

[2] *Memuro Office, Calbee Potato, Inc., Memuro, Kasai, Hokkaido 082-0006, Japan*

[3] *Hokkaido Tokachi Area Regional Food Processing Technology Center, Obihiro, Hokkaido 080-8555, Japan*

[4] *Department of Food Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan*

Received May 28, 2009; Accepted August 31, 2009

By-products from potato processing plants were liquefied and partially saccharified using three commercially available enzymes, followed by ethanol fermentation by yeast and then hydrolysis by glucoamylase. From 12% (w/w) of fresh potato peel, about 20 mg/mL ethanol was formed in the supernatant via amylase, pectinase, or an enzyme complex; this yield was slightly increased with combinations of these enzymes. Supplementation of substandard mash to potato peel (1:1) as a raw material enhanced the amount of ethanol formation, increasing it to approximately 50 mg/mL by the mixture of all three enzymes. After the addition of yeast and glucoamylase to the partially saccharified material, ethanol formation proceeded gradually and slowed in 12 h with the consumption of fermentable sugars. Galacturonic acid derived from pectin was not fermented and remained in the fermented broth. In the by-products, the conversion rate of sugars to ethanol was estimated to be 42.5%.

Keywords: potato peel, substandard mash, ethanol, fermentation

Introduction

Potato is a valuable food crop. Three million tons are produced annually in Japan for a wide range of applications, including cooking, processing, and use as a material for starch. Three hundred thousand tons are used for manufacturing of potato snacks, accounting for 60% of potatoes used for processing. In 2007, sales figures in billions of yen for a variety of potato snacks were 77.5 for potato chips, 54.5 for fabricated potato, and 5.2 for shoestring potatoes. During potato processing, disposal of by-products often poses a serious problem to the manufacturer. The peel removed from the potato tuber prior to slicing and frying is a principal by-product in typical potato chips, and a substandard portion of mashed potato is further loaded in fabricated potato. Potato peel has a high content of phenolic compounds, to which antioxidant activity is attributed (Kanatt *et al.*, 2005; De Sotillo *et al.*, 1994). The main components were identified as chlorogenic acid and caffeic acid in free-form phenolics (Kanatt *et al.*, 2005) and ferulic acid in bound-form phenolics (Nara *et al.*, 2006). Potato peel extract can be used as a natural antioxidant in soy bean oil (Rehman *et al.*, 2004) and has been shown to retard lipid oxidation in radiation-processed lamb meat (Kanatt *et al.*, 2005). Recently, its protective effect against carbon tetrachloride-induced liver injury in rats has been identified (Singh and Rajini, 2008). While banana peel and citrus have been used as media for microbial biomass (Essien *et al.*, 2005) or for enzyme production by fungi (Mamma *et al.*, 2008), most of the by-products from the potato industry are used in animal feed with a combination of other crops (Radunz *et al.*, 2003) or microbial digestion (Ugwuanyi *et al.*, 2008). Plans are being made to use more of the by-products from potato processing. This study investigated the optimal conditions for enzymatic hydrolysis and successive ethanol fermentation of these potato by-products.

Materials and Methods

Raw materials Potato peel and substandard mash obtained from a local plant producing fabricated potato were stored by freezing just before use in the experiments. At this

*To whom correspondence should be addressed.
E-mail: yujioda@obihiro.ac.jp

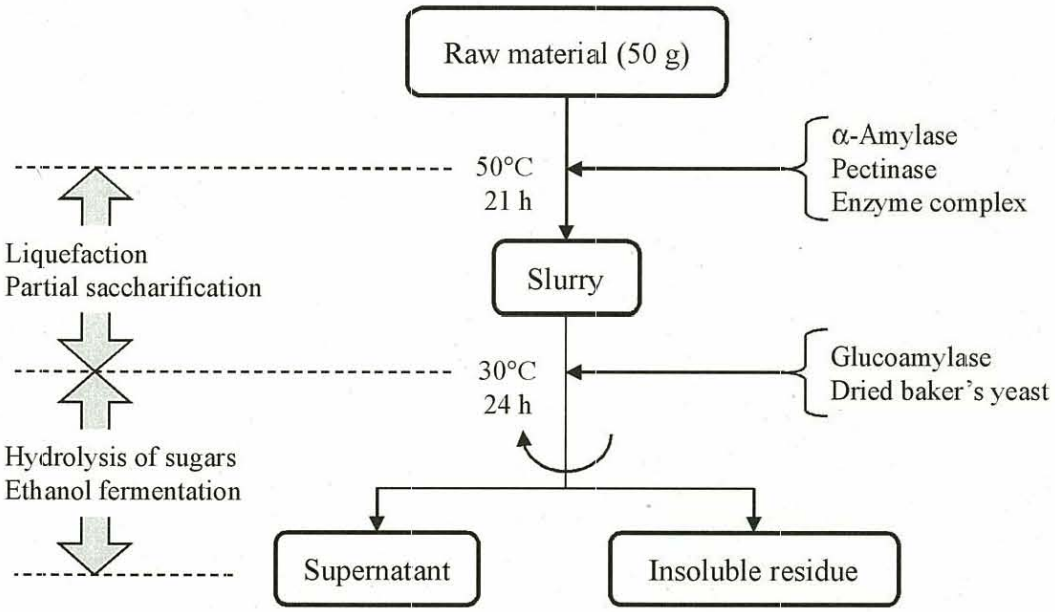

Fig. 1. Procedure outline for treating raw materials from potato processing plants.

plant, the peel is initially removed from the potato tuber using steam to obtain the flesh. After slicing, paste from boiled and mashed potato adheres to a large drum by pressing through five serial rollers and is used for successive processes. The paste that lacks starch content and contains contaminated peel is removed from the drum as substandard mash. The amount of potato peel and substandard mash removed from the potato is about 10% and 3%, respectively.

Enzymes and yeast The commercially available enzyme preparations used were α-amylase (Thermamyl, 120 kilo Novo α-amylase unit [KNU]/g), glucoamylase (AMG 300L, 300 amyloglucosidase unit [AGU]/mL), and an enzyme complex (NS50012, 100 fungal β-glucanase unit [FBG]/g; Novozymes A/S, Denmark) and pectinase (Pectinase HL, 10,000 pectinase unit [PU]/g, Yakult Pharmaceutical Industry, Japan). The yeast *Saccharomyces cerevisiae* used for ethanol fermentation was conventional dried baker's yeast (Super Camellia Dry Yeast, Nisshin Foods, Inc., Tokyo, Japan).

Hydrolysis and fermentation Outline of the standard procedures in the experiments is summarized in Fig. 1.The enzymes, except for glucoamylase, were added to 50 g of raw material as fresh weight, which included potato peel (50 g) or the mixture of potato peel (25 g) and substandard mash (25 g), in a 100-mL sterilized bottle (Fig. 2). After static incubation at 50°C for 21 h, the resulting slurry was cooled to 30°C and inoculated with dried baker's yeast and glucoamylase, which is unstable at 50°C. The slurry was further incubated at 30°C for 24 h with standing and centrifuged to separate the supernatant from insoluble residues. The amount of enzyme and dried yeast cells was determined to be 0.05% and 5.0% (w/w), respectively, based on the dry matter of raw materials, unless otherwise stated.

Analytical methods Ethanol in the supernatant of the slurry was determined using a high-performance liquid chromatograph (LaChrom Elite, Hitachi High-Technologies Corp., Tokyo, Japan) equipped with an RI monitor. The analytical conditions were as follows: column, Shodex KS-801 (8.0 mm × 30 cm, Showa Denko Co., Tokyo, Japan); column temperature 50°C; mobile phase, ultra pure water; injection volume, 20 μL, and flow rate 1.0 mL/min. Soluble sugars were analyzed by thin-layer chromatography (TLC) using

Fig. 2. Mixture of potato peel and substandard mash as raw materials.

TLC silica gel 60, aluminum backed (Merck) with a solvent system of 1-butanol/2-propanol/acetic acid/water (7:5:2:4, v/v) (Watanabe and Oda, 2008). Spots were visualized by spraying the plate with an anisaldehyde-sulfuric acid reagent (Sassalo *et al.*, 2008).

The chemical composition was determined according to the method of the Association of Official Analytical Chemists (Thiex, 2000). The content of neutral detergent fiber enables the estimation of cellulose, hemicellulose, and lignin content. The remaining content excluding the determined components was distinguished as other substances, such as pectin and soluble sugars.

Results

Liquefaction and partial saccharification The chemical composition of the two raw materials is shown in Table 1. The content of all components was higher in potato peel than in substandard mash, excluding starch which was the main constituent of the mash. Compared with amylase and pectinase, addition of the enzyme complex to potato peel resulted in more ethanol formed in the supernatant (Table 2). This enzyme preparation including arabanase, cellulose, β-glucanase, hemicellulase, and xylanase is used to extract components from plant tissue by degrading cell walls. The addition of two or three enzymes further enhanced the ethanol concentration, but only 30 mg/mL was retained because of the limited amount of fermentable substrates. Mixing of substandard mash with potato peel (1:1) stimulated ethanol formation, as expected. In contrast to potato peel, the most effective enzymes for the mash were amylase and pectinase, which contain appreciable amylase activity. As raw potato starch is rarely susceptible to digestion without a previ-

Table 1. Chemical composition of the raw materials of potato.

Item	Potato peel	Substandard mash
Moisture (g/100 g FM)	87.6	77.4
pH	4.9	5.5
Component (g/100 g DM)		
Crude protein	14.3	8.8
Crude fat	1.2	0.2
Crude ash	11.7	3.9
Starch	7.7	49.9
Neutral detergent fiber	31.2	12.0
Other substances	33.9	25.2

FM, fresh matter; DM, dry matter

Table 2. Ethanol formed from raw materials treated with three enzymes.

Enzyme added			Ethanol (mg/mL of supernatant)	
Amylase	Pectinase	Enzyme complex	Potato peel	Potato peel + substandard mash
+	–	–	21.4 ± 1.4	39.2 ± 3.3
–	+	–	21.1 ± 0.9	38.7 ± 1.2
–	–	+	24.6 ± 0.5	32.3 ± 3.8
+	+	–	31.5 ± 1.2	46.0 ± 1.1
+	–	+	26.4 ± 1.0	44.5 ± 2.5
–	+	+	22.6 ± 0.6	42.4 ± 0.9
+	+	+	29.6 ± 2.8	48.6 ± 1.3

Data are shown as the average values ± standard deviations from three independent experiments.

ous gelatinization treatment (Noda *et al.*, 2008), starch in substandard mash was readily hydrolyzed by the enzymatic reaction due to boiling before the mashing process. The most ethanol (48.6 mg/mL) obtained from the mixture of potato peel and substandard mash was accomplished by the combination of all three enzymes. The ethanol concentration proportionally increased with the amount of these enzymes and reached a nearly constant level over 0.05% (Fig. 3).

Sugar hydrolysis and ethanol fermentation Ethanol was formed from the potato peel/substandard mash mixture in successive processes. That is, ethanol was gradually formed during an incubation period of up to 12 h (Fig. 4). Analysis of the supernatant showed that maltotriose derived from starch was efficiently degraded by glucoamylase in 4 h (Fig. 5). Afterwards, fermentable sugars were consumed by the yeast cells and became undetectable. Galacturonic acid derived from pectin was not fermented and remained in the supernatant.

Recovery of ethanol The total sugar content of starch, neutral detergent fiber, and other substances was initially 15.9 g (= [100 − 79.9] × [29.6 + 21.4 + 28.3] / 100) in 100 g of the potato peel/substandard mash mixture as fresh matter (Table 3). If fermentation proceeds with 100% efficiency, 15.9 g of sugars assumed as a polymer of fermentable hexose, $(C_6H_{10}O_5)_n$, is converted to 9.03 g of ethanol, $(C_2H_5OH)_{2n}$. After fermentation, the actual content of ethanol formed was 3.84 g (/100 g), corresponding to 42.5% of the theoretical value. The conversion rate of sugars to ethanol is reasonable because the total sugar in the raw materials includes an appreciable amount of non-fermentable sugars. The content of crude protein and neutral detergent fiber was increased, accompanied by a decrease in the starch content.

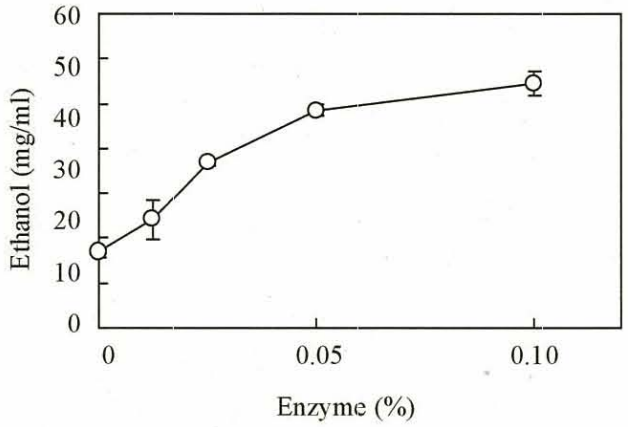

Fig. 3. Effect of enzymes on the production of ethanol from the potato peel/substandard mash mixture. Equal amounts of commercially available amylase, pectinase, and an enzyme complex were added at the same time. Data are shown as the average values ± standard deviations from three independent experiments.

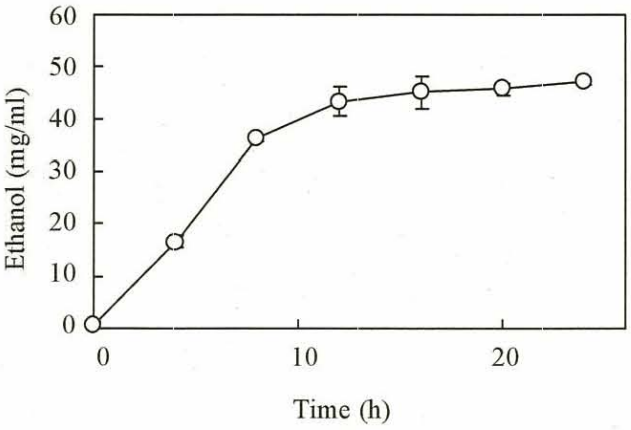

Fig. 4. Time course of ethanol formation from the potato peel/substandard mash mixture. Data are shown as the average values ± standard deviations from three independent experiments.

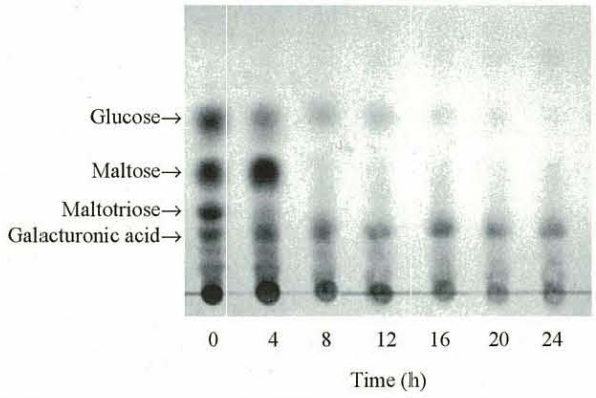

Fig. 5. TLC analysis of sugars in the supernatant of the mixture. The combined supernatant (1 μL) from the mixture in the experiments of Fig. 2 was applied to TLC after three-fold dilution with pure water.

Table 3. Change of chemical composition in the potato peel/substandard mash mixture during saccharification and fermentation.

	Before	After
Moisture (g/100 g FM)	79.9	85.9
Ethanol (g/100 g FM)	< 0.01	3.84
pH	5.2	4.0
Component (g/100 g DM)		
Crude protein	13.6	21.8
Crude fat	0.8	2.0
Crude ash	6.3	10.1
Starch	29.6	6.0
Neutral detergent fiber	21.4	36.3
Other substances	28.3	23.8

FM, fresh matter; DM, dry matter.

Discussion

The use of renewable biofuels as alternatives to fossil fuels is expanding in many countries to reduce the reliance on petroleum and to decrease greenhouse gas emissions (Hill *et al.*, 2006; Lin and Tanaka, 2006). Ethanol, one of the major biofuels, is occasionally called bioethanol to discriminate chemically synthesized ethanol from that produced from sugarcane, corn, and other crops (Gray *et al.*, 2006; Somerville, 2007). However, excessive utilization of crops for ethanol production negatively affects the global food supply. Ethanol production from a renewable source that is unsuitable for food and feed, such as agricultural residues and hardwood species, is required to meet the increasing worldwide demand.

Potato, a principal rotation crop in Hokkaido (the northernmost island of Japan), generates by-products applicable for ethanol production during food processing. Local starch-manufacturing plants use about one million tons of potatoes annually, accompanied by the excretion of pulp corresponding to 10% of fresh tuber (Oda *et al.*, 2002). However, potato pulp cannot fulfill the requirements for ethanol production. High energy must be used for the gelatinization of residual starch by heating prior to enzymatic hydrolysis. Furthermore, fresh potato pulp is generally available only from September to November, during which potato processing mostly takes place. On the other hand, potato peel and substandard mash can be used as raw materials without further heat treatment from processing plants operating daily throughout the year. The resultant residue following fermentation has been supplied to animals as concentrated feed, such as dried distillers grains with solubles (Stein and Shurson, 2009).

The Ministry of Agriculture, Forestry, and Fisheries of Japan has recently set a goal for the production cost of ethanol at 100 yen/L. If substandard mash (3,000 tons) derived from 100,000 tons of fresh tuber is treated by mixing with an equal portion of potato peel (3,000 tons) excreted simultaneously, 292 kL of pure ethanol can be produced in addition to about 800 ton of dried residue for animal feed (Table 3). Yeast cells can be propagated and supplied for the fermentation process as in commercialized manufacturing plants. One liter of ethanol derived from 4.13 kg of the substandard mash and potato peel mixture on a dry basis requires 9.26 g of the total enzymes corresponding to 33 yen assuming the average price of the four enzymes is 4,000 yen/kg. The cost for purchasing enzymes may be compensated by saving on raw material costs, which usually cover 50 to 70% of the total cost for ethanol production from crops (İçöz *et al.*, 2009). Further cost reduction could be accomplished using cheaper enzymes or preparing enzymes by cultivating certain microorganisms secreting these potential enzymes. Therefore, utilization of by-products of potato processing could improve the profits of the potato industry and sustain the development of agriculture in Hokkaido.

Acknowledgements We thank T. Hirai for his technical assistance. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Cooperation of Innovative Technology and Advanced Research in Evolutionary Area).

References

de Sotillo, D.R., Hadley, M. and Holm, E.T. (1994). Potato peel waste; stability and antioxidant activity of a freeze-dried extract. *J. Food Sci.*, **59**, 1031-1033.

Essien, J.P., Akpan, E.J. and Essien, E. P. (2005). Studies on mould growth and biomass production using waste banana peel. *Bioresour. Technol.*, **96**, 1451-1456.

Gray, K.A., Zhao, L. and Emptage, M. (2006). Bioethanol. *Curr. Opin. Chem. Biol.*, 10, 141-146.

Hill, J., Nelson, E., Tilman, D., Polasky, S. and Tiffany, D. (2006). Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proc. Natl. Acad. Sci. USA*, **103**, 11206-11210.

İçöz, E., Tuğrul, K.M., Saral, A. and İçöz, E. (2009). Research on ethanol production and use from sugar beet in Turkey. *Biomass Bioenergy*, **33**, 1-7.

Kanatt, S.R., Chander, R., Radhakrishna, P. and Sharma, A. (2005). Potato peel extract-a natural antioxidant for retarding lipid peroxidation in radiation processed lamb meat. *J. Agric. Food Chem.*, **53**, 1499-1504.

Lin, Y. and Tanaka, S. (2006). Ethanol fermentation from biomass resources: Current state and prospects. *Appl. Microbiol. Biotechnol.*, **69**, 627-642.

Mamma, D., Kourtoglou, E. and Christakopoulos, P. (2008). Fungal multienzyme production on industrial by-products of the citrus-processing industry. *Bioresour. Technol.*, **99**, 2373-2783.

Nara, K., Miyoshi, T., Honma, T. and Koga, H. (2006). Antioxidative activity of bound-form phenolics in potato peel. *Biosci. Biotechnol. Biochem.*, **70**, 1489-1491.

Noda, T., Takigawa, S., Matsuura-Endo, C., Suzuki, T., Hashimoto, N., Kottearachchi, N.S., Yamauchi, H. and Zaidul, I.S.M. (2008). Factors affecting the digestibility of raw and gelatinized potato starches. *Food Chem.*, **110**, 465-470.

Oda, Y., Saito, K., Yamauchi, H. and Mori, M. (2002). Lactic acid fermentation of potato pulp by the fungus *Rhizopus oryzae*. *Curr. Microbiol.*, **45**, 1-4.

Radunz, A.E., Lardy, G.P., Bauer, M.L., Marchello, M.J., Loe, E.R. and Berg, P.T. (2003). Influence of steam-peeled potato-processing waste inclusion level in beef finishing diets: effects on digestion, feedlot performance, and meat quality. *J. Anim. Sci.*,

81, 2675-2685.

Rehman, Z.U., Habib, F. and Shah, W.H. (2004). Utilization of potato peels extract as a natural antioxidant in soy bean oil. *Food Chem.*, **85**, 215-220.

Sassalo, G.L., de Soua, L.M., Cipriani, T.R. and Klacomini, M. (2008). TLC of carbohydrates. In "Thin layer chromatography in phytochemistry", ed. by M. Waksmundzka-Hajnos, J. Sherma and T. Kowalska. CRC Press, Boca Raton, pp. 255-276.

Singh, N. and Rajini, P.S. (2008). Antioxidant-mediated protective effect of potato peel extract in erythrocytes against oxidative damage. *Chem. Biol. Interact.*, **173**, 97-104.

Somerville, C. (2007). Biofuels. *Curr. Biol.*, **17**, R115-R119.

Stein, H.H. and Shurson, G.C. (2009). Board-invited review: The use and application of distillers dried grains with solubles in swine diets. *J. Anim. Sci.*, **87**, 1292-1303.

Thiex, N.J.W. (2000). Chapter 4. Animal Feed. In "Official Methods of Analysis of the AOAC International, 17th ed.", ed. by W. Horwitz. AOAC International, Gaithersburg, MD.

Ugwuanyi, J.O., Harvey, L.M. and McNeil, B. (2008). Diversity of thermophilic populations during thermophilic aerobic digestion of potato peel slurry. *J. Appl. Microbiol.*, **104**, 79-90.

Watanabe, T. and Oda, Y. (2008). Comparison of sucrose-hydrolyzing enzymes produced by *Rhizopus oryzae* and *Amylomyces rouxii*. *Biosc.i Biotechnol. Biochem.*, **72**, 3167-3173.