UTILIZATION OF ALKALINE POTATO PEEL WASTE BY FERMENTATION. AMYLASE PRODUCTION BY *ASPERGILLUS FOETIDUS* NRRL 337, AND ALCOHOLIC FERMENTATION

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ABSTRACT

Aspergillus foetidus NRRL 337 was cultured on hydrochloric acid neutralized waste from the dry caustic potato peeling process. Alpha-amylase yields of 26-40 SKB units/ml and maltase yields of 6 to 10 units/ml were obtained. The fermented mixture filters very easily to yield an amylase containing liquor and mycelium. Alcoholic fermentation of additional peel waste using the above for amylolytic conversion gave alcohol yields of up to 90% on a stoichiometric carbohydrate basis. This work was done to demonstrate the utility of dry caustic peel waste as a starting material for fermentation processes.

INTRODUCTION

The infra-red potato peeling process has found large-scale commercial plant application (1). The process cuts caustic usage, reduces peel loss, and results in a more concentrated alkaline peel residue. The peel is used as cattle feed after the pH is reduced.

Commercially, 20,000 pounds of potatoes are peeled per hour per peeling line, producing 2,500 pounds of alkaline peel. One large potato processing plant operates eight such peeling lines. About 240 tons of alkaline peel are produced per day, 300 days a year, and many thousands of cattle are fed from this waste. The nearly year-round supply, the large volume, and the potential increase in processing volume make a process for upgrading the alkaline peel waste interesting and desirable.

The work to be described is an attempt to upgrade the peel by fermentation procedures with no, or only very limited, dilutions and with complete utilization. Alcoholic fermentation was chosen because the distillery slops from potato fermentation are a recognized cattle feed and consequently there is no waste. Additionally, the distillery slops are a more balanced feed than the original peel waste.

In order to carry out an alcoholic fermentation, the starch in the potato peel has to be converted to fermentable sugars. We selected the enzymatic conversion by amylase produced by *Aspergillus foetidus*, NRRL 337, to avoid the cost of malting. To our knowledge, this organism has never been grown on potato peel waste. It had been proposed that these fungal amylases could replace malt in some applications hence this could also result in another marketable product.

The necessity for neutralization is peculiar to the utilization of the alkaline peel waste. The 2 pounds of sodium hydroxide which are used per 1,000 pounds of potatoes are contained in about 125 pounds of peel. The alkalinity of the peel waste determined by titration was 1.4% expressed as NaOH. However, 55 to 89% of the alkalinity of the peel waste received was actually carbonate.

[1]Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Berkeley, California 94710. Received for publication March 26, 1973.

Sulfuric acid can be used to at least partly replace the HCl used herein for neutralization. However, when the final distillery slops are fed to cattle, a large sulfate content may be objectionable, whereas NaCl is not.

Material and Methods

Three lots of alkaline peel from commercial plant operations were received and held at 34 F (1.1 C). The composition of these three lots is shown in Table 1. The accounted-for material is in all cases about 85-90%, which is an acceptable value, since the analytical methods do not include tests for organic acids and some of the hemicellulose. Furthermore, the factor 6.25 to convert nitrogen to protein has not been established for potato peelings.

The material was very viscous and lumpy, with an odor of ammonia. The starch was gelatinized, showing no birefrigence in a polarizing microscope except for some ungelatinized small potato pieces.

Submerged culture of A. foetidus NRRL 337

The alkaline waste was thoroughly mixed in a 5 quart Hobart mixer and diluted to 5% solids. The media were then acidified with HCl to pH 5.5 and ammonium and phosphate salts were added. Various combinations of autoclaving were tried to find the optimum heat treatment. Finally, ammonium bifluoride and calcium carbonate were added to the media. Table 2 shows the final composition of the medium.

Cell breakdown

It was found that in the raw peel waste a part of the starch, although gelatinized, was still inside cells and the enzymes apparently could not reach it to hydrolyze it. To break down the cell walls autoclaving — either before or after neutralization, or both — and shearing in an amylograph were tried.

Conversion of peel waste and alcoholic fermentation

One kg of peel waste was mixed and acidified to pH 6 with 6N HCl in a Hobart mixer. The material was transferred to a Fernbach flask, stoppered, autoclaved or sheared, and cooled to 67 C. Unfiltered mold broth was mixed in the ratio of one part mold broth to 9 parts potato waste and the mixture was agitated at 62 to 67 C for 20 min. The flask was cooled to 30 C and bakers' yeast (10 g per liter, dispersed in 20 g of water) was added. The Fernbach flasks were topped with tubed stoppers to allow the CO_2 to escape. The fermentations were carried on for 68-72 hrs at 30-31 C while shaking at 75 strokes per minute. An aliquot of the fermented mash was distilled, and the alcohol determined in the distillate by the refractometric method (4). The α-amylase was determined by the method of Sandstet et al (7). The maltase activity was determined according to Tsuchiya et al (8). Hydrolyzable carbohydrates were determined by 1 normal trichloroacetic acid at 120 C for 90 min and are reported as glucose.

Viscosity

The viscosity of the peel waste was determined at constant temperature and different shear rates using a Brockfield viscometer. The temperature coefficient of viscosity at a given shear rate was determined by means

TABLE 1. — *Composition of Alkaline Potato Peel Waste from Commercial Plant Operations*

	A As rec'd. %	B As rec'd. %	C As rec'd. %
Solids	16.0	14.6	15.5
N	0.22	0.33	0.34
N x 6.25	1.35	2.06	2.12
Crude fat	0.05	0.05	0.18
Crude fiber	0.56	0.81	0.81
Ash	2.23	2.55	2.86
Na	0.70	0.76	0.90
P	0.037	0.05	0.05
Hydrolyzable carbohydrates	10.15	7.59	7.83
Alcohol-soluble sugars	0.07	0.29	0.09
pH	12.7	11.9	12.1
Alkalinity as NaOH	1.40	1.29	1.39

TABLE 2. — *Aspergillus Foetidus NRRL #337*

Final composition of growth media (wt. percent)	
Potato peel, solids	5.0
Ammonium sulfate	0.4
Monoammonium phosphate	0.1
Ammonium bifluoride	0.02
Calcium carbonate	0.5
Polypropylene glycol 2025 defoaming agent	0.05

of an amylograph. The dependence of viscosity on shear rate was determined by the method of Metzner (6).

RESULTS

Titration of the peel waste

Fig. 1 shows a titration curve for peel waste A in Table 1 using 1N HCl.

A. foetidus NRRL 337 culture

The results of some typical *A. foetidus* cultures are shown in Table 3. The first three lines show conditions tried other than the optimum while the fourth line shows a typical run at optimum conditions. Only a few analyses of maltase were done. They showed values in the range of 6-10 units/ml in samples with high (25-40 SKB units/ml) α-amylase activity. One unit of maltase activity is that amount of enzyme which hydrolyzes 1 mg of maltose monohydrate in 1 hr at 30 C. One SKB unit is that amount of α-amylase which dextrinizes 1 g of β-amylase treated starch in 1 hr at 20 C. These values agree with those of Tsuchiya et al (8) of 8 units/ml maltase in the presence of 9.05M $CaCO_3$ to control pH. Tsuchiya et al achieved yields of up to 18 units/ml with

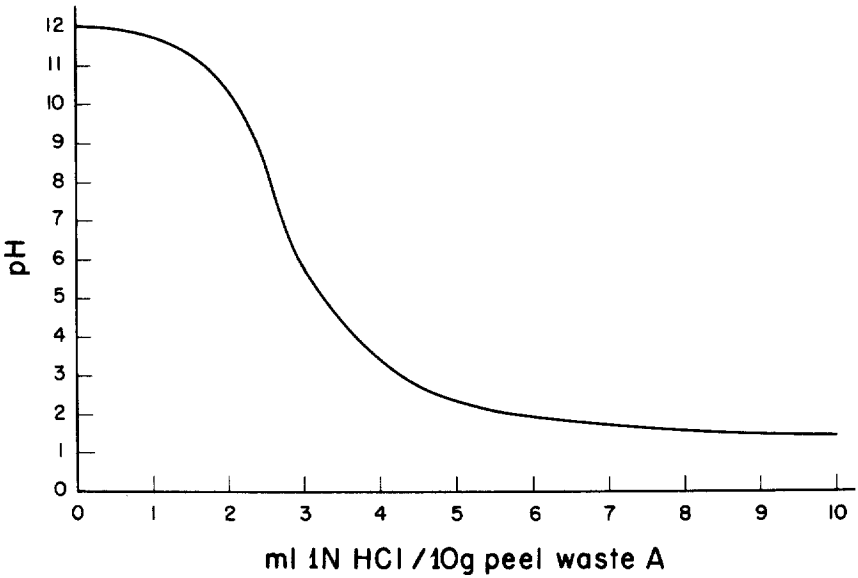

FIG. 1.—Titration curve of alkaline peel waste with 1N HCl.

TABLE 3.—*A. foetidus NRRL #337 fermentation.*

	Typical results			
	Activity of α-amylase, SKB units[1]			
	Inoculate	1st Transfer	2nd Transfer	3rd Transfer
	(Maltose Medium)			
1. Potato peel solids + water flask plugged during fermentation	5.9	2.6	0.4	0.0073
2. 0.4% $(NH_4)_2SO_4$ & 0.1% $NH_4H_2PO_4$ added[2]	.37	.69	40	21
3. Plug replaced by double milk filter disk, 0.08% $CaCO_3$ added[2]	6	35.5	6	——
4. 0.02% $NH_4F.HF$ added ..	18	25	27	28

[1] 1 SKB unit is the amount which dextrinizes 1g of β-amylase treated starch in 1 hour at 20C.
[2] Inconsistent results. Contaminated with bacteria at times.

$CaCl_2$ or other agents to control pH. However, since 8 units/ml is sufficient to saccharize the peel waste in 20 min at 65 C, efforts to increase yield were not pursued.

TABLE 4.—*Alcoholic fermentation of saccharized potato peel waste.*

Pretreatment	Typical results		
	Alcohol produced, ml per 100 g peel waste	Yield based on alcohol (%)	Yield based on hydrolyzable slops (%)
Neutralized, to pH 6 with HCl	2.65	51.7	—
Neutralized, autoclaved[1] 1 hr.	4.02	78.5	—
Neutralized, autoclaved[1] 2 hr.	4.33	84.5	80.6
Autoclaved[1] 1 hr., neutralized, autoclaved[1] 1 hr.	3.78	73.8	—
Neutralized, autoclaved[2]	3.88	75.5	78.9
Neutralized, autoclaved twice[2]	3.33	63.9	71.6
Neutralized, sheared	4.33	84.5	83.4
Sheared,[4] neutralized, sheared[4]	4.51	87.9	84.4
Theoretical	—	100	100

[1]121C.
[2]½ hr. at 121C, then ½ hr. at 152 C.
[3]1 hr. at 121C, then 1 hr. at 152 C.
[4]1 hr. in amylograph.

Alcoholic fermentations

Results of some typical yeast fermentations are shown in Table 4. Results from two independent determinations of the yield were fairly consistent. Table 5 shows the composition of a typical slope. The comments about the analyses in Table 1 also apply to this table.

Viscosity

The dependence of shear stress on shear rate was determined at a constant temperature and as a function of temperature using sample C in Table 1. The data are correlated by:

$$\text{tau} = 1.35 \left(-\frac{du}{dr}\right)^{.273} \qquad (1)$$

Where tau = shear stress (lbs. force/ft^2)

$-\left(\frac{du}{dr}\right)$ = shear rate (sec^{-1})

Furthermore, the apparent viscosity, μ_a, of the material is:

$$\mu_a = 1.35\left(-\frac{du}{dr}\right)^{-.727} \frac{\text{lb. force}}{\text{sec.-ft.}} \qquad (2)$$

Similarly, using the amylograph it was found that the activation energy for viscosity of the peel waste was + 2050 kcal/g mole.

$$\text{Therefore, } \mu = \mu_{25}\exp\left[\frac{-2050}{R}\left(\frac{1}{T} - \frac{1}{298}\right)\right] \qquad (3)$$

where μ = viscosity at T
μ_{25} = viscosity at 25 C
T = Temp. (°K)

$$R = \text{gas constant} = 1.987 \frac{\text{g cal}}{\text{g mole} = {}^\circ\text{K}}$$

TABLE 5. *Composition of typical slops*

Dry solids	7.0% % of dry solids
N	3.3
N x 6.25	20.8
Crude fiber	7.8
Ash	25.8
Hydrolyzable carbohydrates	29.0
Na	6.8
Ca	0.4
Cl	7.8
P	0.5
P_2O_5	1.2
pH = 4.5	

It should be emphasized that this waste is highly variable in composition, and these equations should be used only to extrapolate measured data for a given waste to give a reasonable estimate for pump requirements and the like. Raw values of apparent viscosity varied from a few hundred centipoises at 70 C and high shear to close to a million centipoises at 20 C and low shear rates.

Discussion of Results

As can be seen from the results, an adequate supply of oxygen was necessary for successful mold growth. Even with all nutrients present and bactericides present, good growth was not obtained in cotton-stoppered, agitated flasks, while those covered only with more permeable milk filters gave good results under the same conditions. Organic nutrients such as yeast extract and corn steep liquor do not appear necessary for optimal α-amylase development.

It should be noted that the yields of α-amylase reported here are higher than any previously reported in the literature (2, 3, 5, 7). The highest yield previously reported was 23 SKB units per milliliter by Hanson et al (3). We were consistently able to obtain yields of 25-40 SKB units per milliliter. The only explanation we have for the higher yields is the use of potato rather than some other growth medium.

An added benefit of this medium is the ease of filtration when the medium was autoclaved twice before addition of the mold. Typically one could filter 600 ml in a 15 cm Buchner funnel in less than two minutes. This would be a very valuable property in any commercial application to the production of amylases or other mold fermentation products.

Also of importance in industrial application is that the transfers were typically 1 part to 100 parts of new medium. Therefore, from an initial culture of 500 ml (1 pint) grown from a slant, one should be able in three transfers to obtain 500,000 liters (125,000 gal) with an α-amylase activity of 25-40 SKB units/ml.

It was found that pretreatment of the peel waste was the single most important factor in increasing alcohol yield. This was apparently due to there being a large number of intact cells present which could not be attacked easily by the amylolytic enzymes. As can be seen from Table

4, the best treated waste gave alcohol yields 70% higher than the untreated waste. There seemed to be a limit to the severity of heat treatment possible. Samples autoclaved at 152 C gave lower yields than those autoclaved at 144 C. It was also found that autoclaving before neutralization lowered the yield. Longer shearing times in the amylograph, on the other hand, gave the best alcohol yield. This is due, of course, to the chemical destruction of some starches by high temperature or high pH while the purely physical action of the shearing would cause much less destruction. It is visualized that a very high shear rate for a short time could give high cell destruction at a reasonable cost, especially considering that the viscosity decreases markedly under high shear rates for a pseudoplastic material such as this. The cost of shearing versus the loss in alcohol if the untreated waste is used suggests an economic optimization to determine the amount of shearing that should be done.

The saccharization of the peel waste with the mold solution presented no problems. It was done on a batch scale in a 2800 ml flask with agitation or in an amylograph, and on a continuous scale by pumping the mold solution-peel waste mixture through a 1 inch polyethylene tube immersed in a 65 C bath.

Any scheme for the use of peel waste as a fermentation raw material must consider, of course, the economics of the processes. The cheapest raw material currently avavilable for fermentation is cane molasses at $30 per ton or $2.85 per 100 lb sugar. This would be equivalent to a value of about $5.70 per ton for the potato peel waste based on 200 lb per ton of fermentable sugars after hydrolysis. The figures are not directly comparable, as acidification and starch conversion add to the processing cost of alkaline peel. If HCl can be obtained at $0.02 per lb, neutralization to pH 6 would cost $0.48 per 100 lb of fermentable sugars and neutralization to pH 5, $0.60 per 100 lb of fermentable sugars. On the other hand, molasses yields a fermentation by-product of no value, whereas the by-product from peel waste conversion is an excellent cattle feed. Therefore, if peel waste can be obtained for $5 per ton or less, this process should be immediately competitive. Higher peel waste prices would necessitate a closer economic analysis of by-proluct values, but would not preclude this process.

The cost of acid for neutralization suggests the desirability of recycling waste liquors or the use of CO_2 for neutralization. The feasibility of recycling would depend on a careful economic analysis. Neutralization with CO_2 by several methods was tried. It was found that a pH lower than the bicarbonate endpoint (pH 8.2) could not be obtained. However, HCl or H_2SO_4 could be added to this solution at pH 8.2 and save some of the cost of neutralization.

Conclusions

The use of the peel waste from dry caustic peeling of potatoes for the production of alcohol and high-grade cattle feed appears to be a viable alternative to its present use as a cattle feed. It also appears to be a promising media for the production of amylolytic enzymes because of its high yields of α-amylase and ease of filtration. A further study of increasing the yield of maltase should be undertaken. A careful economic

study of both processes in a commercial situation taking into account geographic and other local factors is needed.

Literature Cited

Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

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