Protein Enrichment of Sweet Potato Residue with Amylolytic Yeasts by Solid-State Fermentation

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Starchy agricultural wastes were inoculated with amylolytic yeasts for protein enrichment by solid-state fermentation. The moisture content of substrate was 65–69%, and water activity was equivalent to 0.98–0.99. The optimum conditions for protein enrichment were initial moisture content 65%, initial pH 4.5, a 1:1 mixture of ammonium sulfate and urea was incrementally added to the ferment with 1% added at zero time, 1% added at 24 h, and 0.5% added at 48 h, and incubation with amylolytic yeasts (1.0×10^{10}/100 g substrate) at 30°C for 2–3 days. The final product contained 16.11–20.82% protein.

INTRODUCTION

The protein demands for direct human consumption and animal feeding will inevitably increase in the next two decades.[1] In 1984, Taiwan imported 1.34×10^6 tons soy beans from the United States, which cost 4.38×10^8 U.S. dollars.[2] Consequently, it is urgent to develop local protein resources with renewable raw materials for animal feed. Considering the cost, a starchy material such as the sweet potato might be a good substrate for protein enrichment for two reasons. First, Taiwan has a large cultivation area (27,791 ha) for sweet potatoes, and the annual production is usually very high, e.g., 4.24×10^5 tons in 1984. Second, the sweet potato can be easily converted to biomass by a great number of fast-growing microorganisms.[1,3]

In order to be economically competitive, the bioconversion of starchy materials into protein should be able to be performed at the rural level. Solid-state fermentations can achieve this purpose by reducing the cost of growing microorganisms, improving the *in vitro* rumen digestibility, and increasing the protein and fat contents of starchy or cellulosic materials.[1,4,5] In this article we report our studies on the possibilities of enriching the protein content of sweet potato residue by the solid-state fermentation process.

MATERIALS AND METHODS

Sweet Potato Residue

Sweet potato residue was purchased from the local market in Taiwan. It contains 14.0–16.1% moisture, 2.32% crude protein, 3.6% ash, 18.1% crude fiber, and 65.4% carbohydrate.

Culture Media and Culture Conditions

Liquid Medium

The tested organism was cultivated in liquid shaking culture at 30°C for 2 days. Soluble starch (0.4%) and yeast extract (0.2%) were used as the carbon and nitrogen sources, respectively.

Solid-State Medium

Sweet potato residue (100 g), nitrogen source [$(NH_4)_2SO_4$, urea, or their mixture] (1–2.5 g), KH_2PO_4 (1 g), and yeast cells (or spores of *Aspergillus*) (1.0×10^9) were mixed thoroughly, and the substrate was adjusted to pH 4.5 with H_2SO_4. The solid-state medium was incubated at 30°C for 2–3 days.

Tested Organism

Pichia burtonii CBS 6141, *Lipomyces starkeyi* CBS 1804, and *Schwanniomyces castllii* CBS 2863 were provided by P. Galzy, ENSA, France. *Saccharomyces diastaticus* IFO 1015 STA 1 (D), *S. diastaticus* IFO 1046 STA 1, STA 2 (D), *S. diastaticus* ATCC 28339 ∝mel 1 DEX MAL, SUC (H), *Saccharomyces* sp. IFO 1426 (D), *S. cerevisiae* IOB 5162 ∝leu 2, ura 1 STA 1 (H) were provided by W. H. Wang, Research Institute for Wines, Taiwan Tobacco and Wine Monopoly Bureau. *Aspergillus niger* NTU-AM-1, *A. niger* NTU-AM-2, and *A. niger* NTU-AM-3 were obtained from the collections of our laboratory. All of them have amylolytic activities.

Chemical Analysis

Moisture Content

Fermented sweet potato residue was dried at 60°C under vacuum for 8–12 h until its weight remained constant. The

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weight difference after drying was considered the moisture content.

Water-Holding Capacity

Sweet potato residue was soaked in water at 20°C for 24 h and centrifugated at 14,000*g* for 1 h, the supernatant was discarded, and the amount of moisture of the residual pellet was determined as water-holding capacity.[6]

Bulk Density

The dry weight or wet weight of sweet potato residue per unit volume (1 cm^3) was the bulk density in dry weight or wet weight, respectively.[7]

Water Activity

Samples with different moisture contents were placed in a sealed container at 25°C, and water activity was determined by a hygrometer.[8]

Soluble Nitrogen

Sample was extracted with 5 times volume of distilled water and shaken for 20 min. Soluble nitrogen was determined directly by the Kjeldahl method.

Protein Content

Protein content was calculated by 6.25 times the difference between total nitrogen and soluble nitrogen of sample.

Total Nitrogen, Total Sugar, Ash, and Crude Fiber Contents

Total nitrogen was determined by the Kjeldahl method, total sugar was determined by the phenol-H_2SO_4 method, crude fiber was determined by ash-free residue digested with 1.25% NaOH and 1.25% H_2SO_4, and ash content was determined by heating at 550–600°C for 5 h.[9]

RESULTS

Physical Properties of Sweet Potato Residue

The water-holding capacity of sweet potato residue was 72%. The moisture content of the substrate was between 65 and 69%, which was lower than the water-holding capacity. The water activity of the substrate was 0.98–0.99. Consequently, the substrate was suitable for the growth of aerobic microbes to enrich the protein content.

The relationship between the bulk density and moisture content of sweet potato residue is shown in Figure 1. The bulk density in wet weight increased with the increase of moisture content, but the bulk density in dry weight was the reverse. The bulk densities were 0.51–0.58 and 0.18–0.19 g/cm^3 in wet weight and dry weight, respectively.

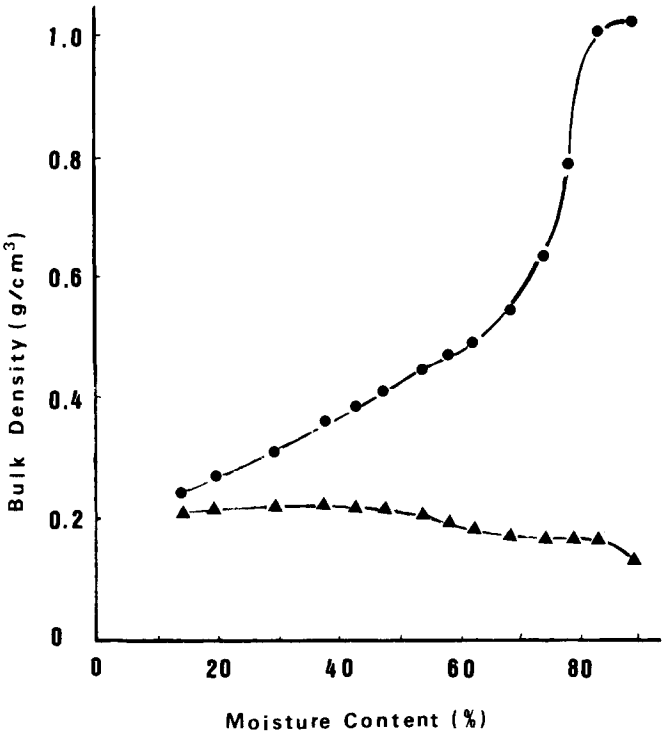

Figure 1. Bulk density of sweet potato residue at different moisture contents: ●——●, wet-weight basis; ▲——▲, dry-weight basis.

Solid-State Fermentation

A home-made column fermentor (6.3 cm i.d., 6.8 cm o.d., height 70 cm) surrounded by a water jacket was used in this study. During the fermentation, the medium was agitated once a day.

Initial Moisture Content

Pichia burtonii CBS 6141 was inoculated into the solid-state medium with the initial moisture ranging from 50 to 74% and incubated at 30°C for 3 days. After culture, it was found that the moisture content of the final product increased 4.0–11.5%. The optimum condition of the maximum value of protein enrichment (4.26% on a dry-weight basis) was at the initial moisture content of 65% and final moisture content of 69%.

If the initial pH of the substrate was 4.5, then the final pH of the product lay in the range of 3.5–4.9. Due to such low pH values in the solid-state fermentation process, the bacterial contamination will be minimized. After fermentation, the bacterial count was only about 10^3 g^{-1} of dry-weight substrate. The bulk density in wet weight increased progressively during the fermentation. The final bulk density increased 65–210% of initial bulk density. The initial and final bulk densities for the highest protein enrichment of sweet potato residue were 0.56 and 1.32 g/cm^3, respectively.

Selection of Tested Organisms

The results of protein enrichment with different amylolytic organisms are shown in Table I. Protein enrichment with *Saccharomyces* sp. IFO 1426 (D), *S. diastaticus* IFO 1046 (D), and *A. niger* NTU-AM-1 was about 5%; that with *S. diastaticus* ATCC 28339 (H), *P. burtonii* CBS 6141, *A. niger* NTU-AM-2, and *A. niger* NTU-AM-3 was between 3.0 and 3.6%; and that with *S. diastaticus* IFO 1015 (D), *S. cerevisiae* IOB 5162, and *S. castellii* CBS 2863 was less than 3.0%. Therefore, *Saccharomyces* sp. IFO 1426 (D) was selected for further study.

Nitrogen Source

The different effects of protein enrichment of sweet potato residue when $(NH_4)_2SO_4$, urea, NH_4NO_3, or a mixture of $(NH_4)_2SO_4$ and urea was used as the nitrogen source are shown in Table II. A 1:1 mixture of ammonium sulfate and urea as nitrogen source gave rise to the highest protein enrichment (14.04%), a 2:1 mixture the second (13.75%), and a 3:1 mixture the third (12.03%). However, if $(NH_4)_2SO_4$, NH_4NO_3, or urea was used solely as the nitrogen source, the protein enrichment was only 4.75–6.65%. In addition, using the mixture of ammonium sul-

Table I. Protein enrichment with different microorganisms by solid-state fermentation.

Tested strain	Initial moisture (%)	Final moisture (%)	Protein enrichment (mg/g)	Initial pH	Final pH	Total carbohydrate decrease (mg/g)	Weight loss (%)	Bulk density increase (%)
Saccharomyces sp. IFO 1426	66.4	67.6	50.5	4.50	3.52	508.5	14.12	3.00
Saccharomyces diastaticus IFO 1046	66.5	69.1	51.0	4.75	2.95	541.0	14.31	3.56
S. diastaticus IFO 1015	66.0	67.5	26.7	4.86	2.97	593.5	10.88	2.29
S. diastaticus ATCC 28339	66.8	69.0	30.0	4.82	3.90	540.0	11.60	5.83
Schwanniomyces castellii CBS 2863	65.5	67.0	23.5	4.90	2.90	248.0	4.10	14.53
Pichia burtonii CBS 6141	65.6	67.5	29.1	4.60	2.81	302.0	4.65	5.25
S. cerevisiae IOB 5162	65.6	68.9	21.1	4.60	3.40	444.3	4.08	6.12
Aspergillus niger NTU-AM-1	70.8	75.6	48.0	3.64	3.34	490.0	17.36	8.00
A. niger NTU-AM-2	74.5	77.7	36.0	4.01	2.92	461.2	13.99	13.64
A. niger NTU-AM-3	70.6	71.3	30.1	4.10	3.28	493.0	10.54	8.86

Table II. Effect of nitrogen source on protein enrichment of sweet potato residue.[a]

Item	Fermentation time (h)	$(NH_4)_2SO_4$	Urea	NH_4NO_3	Ratio of $(NH_4)_2SO_4$ to urea 1:1	2:1	3:1
Moisture content (%)	0	68.69	71.04	69.57	70.34	69.68	69.96
	24	69.19	72.35	69.39	71.07	70.70	70.60
	48	70.09	74.95	69.71	70.21	70.90	70.93
	72	69.62	75.46	70.56	69.94	70.52	71.10
Protein content (%)	0	6.08	6.05	6.05	6.01	5.94	5.99
	24	8.12	9.72	8.37	9.52	8.72	8.64
	48	9.58	10.12	9.24	12.01	10.13	9.87
	72	11.62	12.70	10.75	14.04	13.75	12.03
pH	0	4.7	4.7	4.7	4.7	4.7	4.7
	24	3.2	7.5	3.3	3.5	3.3	3.3
	48	3.1	8.1	3.5	3.5	3.5	3.5
	72	3.1	7.9	3.5	3.5	3.8	3.8
Ash content (%)	0	3.52	3.43	3.41	3.56	3.61	3.59
	24	3.55	3.67	3.53	3.73	3.77	3.83
	48	3.65	3.98	4.02	3.99	3.95	4.04
	72	4.24	4.26	4.18	4.37	4.37	4.37

[a] Fermentation conditions: each gram of substrate inoculated with *Saccharomyces* sp. IFO 1426, 4×10^7 cells, supplemented with 2.5% nitrogen source and incubated at 30°C.

fate and urea from a 1:1 to a 3:1 ratio as the nitrogen source could prevent the pH decline during the fermentation.

The weight of substrate dropped significantly during the fermentation. The values were between 10.54 and 17.36% in 3 days incubation at 30°C. However, this result was consistent with the theoretical value of increasing the ash content of substrate.

Method of Nitrogen Addition

The effect of nitrogen addition on the protein enrichment of sweet potato residue is shown in Table III. A 1:1 or 2:1 mixture of ammonium sulfate and urea incrementally added to the ferment with 1% added at zero time, 1% added at 24 h, and 0.5% added at 48 h had higher protein enrichment than just nitrogen addition at zero time.

Inoculum Cell Size

Each gram of substrate inoculated with 1.0×10^7 cells of *Saccharomyces* sp. IFO 1426 could get 16.11% protein of product in 3 days incubation. While the inoculum cell size increased to 1.0×10^8 cells, the product increased to 20.82% protein.

DISCUSSION

Solid-state fermentations are distinguished from submerged cultures by the fact that microbial growth and product formation occur at or near the surfaces of solid materials with low moisture content.[10] Due to the low moisture content of substrate in solid-state fermentation, the bacteria contamination was not so severe,[11] and the microbes used in solid-state fermentation were limited in a large number of filamentous fungi, some yeasts, some actinomycetes, and few bacteria.[4] The water-holding capacity of a sample could be used as an index of aerobiosis.[6] The moisture content of sweet potato residue used in solid-state fermentation was 65–69% and lower than the water-holding capacity. The condition was suitable for the growth of aerobic microbes. The bulk density on a wet-weight basis increased very sharply when the moisture content was higher than 62%. This phenomenon was very similar to the adsorption isotherm of sweet potato residue.[12] The water was less firmly bound when the moisture content was higher than 62%. At the higher level of moisture content, the multilayer adsorption, the uptake into pores and capillary spaces, and mechanical entrapment of water were very important for microbial growth.[13] In general, gas exchange depends on the bulk density and water-holding capacity of the substrate. The higher the bulk density and water-holding capacity, the slower the thermal diffusibility, oxygen transfer, and microbial transmission observed.[6] During fermentation, the moisture content of substrate increased, which might be due to the production of metabolic water of fungi, as had been observed in the spawn of mushroom[14] and sugar beet residue.[15]

Substrates traditionally used in solid-state fermentation are rice, wheat, millet, barley, corn, and soybeans. However, agricultural waste such as sweet potato residue might also be a good candidate because of its abundant supply and reasonable cost. Sweet potato residue contains 2.32% protein and 65.4% total carbohydrate and is, in itself, not a good source of protein for animal feeding. However, it could be enriched with protein by using amylolytic yeast by a solid-state fermentation process. In order to enrich the protein content from 4–6 to 18–20%, an additional 2.5%

Table III. Effect of nitrogen supplement on protein enrichment.[a]

Item	Fermentation time (h)	$(NH_4)_2SO_4$–urea ratio 1:1		$(NH_4)_2SO_4$–urea ratio 2:1	
		Added at time 0 with 2.5%	Added at 0, 24, and 48 h with 1, 1, and 0.5%	Added at time 0 with 2.5%	Added at 0, 24, and 48 h with 1, 1, and 0.5%
Moisture content (%)	0	62.37	60.42	62.28	59.93
	24	60.93	58.36	62.01	59.05
	48	59.75	61.38	59.83	60.26
	72	61.43	65.81	62.41	66.06
Protein content (%)	0	5.93	5.54	5.43	5.46
	24	8.14	9.84	8.51	9.03
	48	11.16	12.61	11.25	12.49
	72	15.07	17.99	15.05	16.84
pH	0	4.9	4.9	4.9	4.9
	24	4.5	4.5	4.5	4.3
	48	4.3	4.1	4.3	4.1
	72	4.1	3.9	3.9	3.7
Ash content (%)	0	3.66	3.60	3.45	3.70
	24	3.93	3.89	3.80	3.90
	48	4.06	4.02	4.09	4.12
	72	4.43	4.30	4.31	4.47

[a] Fermentation conditions: Each gram of substrate inoculated with 4×10^7 cells *Saccharomyces* sp. IFO 1426 and incubated at 30°C.

nitrogen supplement is necessary theoretically. In this study, we found that the fractional supplement of nitrogen could result in a higher protein enrichment than supplement added only at zero time. These might be due to the maintenance of substrate pH, lowering the nitrogen source inhibition, and increasing nitrogen efficiency.

Each gram of substrate with 1.0×10^7–1.0×10^8 cells could produce a product containing 16.11–20.82% protein. The ORSTOM (Office de la Recherche Scientifique et Technique Outre-Mer, Paris) group indicated that the inoculum cell size of cassava starch in solid-state fermentation was 2.6×10^7 conidia of *Aspergillus niger* per gram of substrate. The starchy material containing 2–5% protein was transformed into enriched product containing 18–20% protein.[1]

Saccharomyces does not produce the fungal toxin of *Aspergillus*.[16] Therefore, the commercial utility of protein enrichment of sweet potato residue with *Saccharomyces* sp. IFO 1426 by solid-state fermentation for animal feed appears to be promising.

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