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FERMENTATION UTILIZATION OF CASSAVA. THE BUTYL-ACETONIC FERMENTATION

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The cassava plant belongs to the family *Euphorbiaceae* and is botanically known as *Manihot utilissima* Pohl. It is also called tapioca or manioc although the word tapioca is often used to designate certain forms of cassava products. The cassava is a plant possessing quite unusual characteristics. It has no known pests nor enemies. It grows in most soils, resists extreme droughts, and propagates easily although its growth is restricted to tropical regions. The plant itself is a perennial shrub which attains a height of six to twelve feet at the age of one year. At the base of its stem it produces a cluster of long fleshy roots. The starch content of the fresh cassava root is 25 to 30 per cent; these roots furnish the cheapest source of starch known.

American technologists who early visited the Philippine Islands were filled with enthusiasm over the cassava. In the Philippines the yield of cassava was reported by Mendiola (1) to be 16,100 to 38,300 kg. per hectare (7.18 to 17.3 tons per acre); in Java yields as high as 50,000 to 55,000 kg. per hectare (22.3 to 24.7 tons per acre) have been obtained. However, in spite of its promise, up to the present time no industrial utilization has been made of cassava except for the manufacture of starch. Cassava starch is found in the market throughout the world because it can undersell any other starch. This is a disturbing competitive factor in the United States in attempts to increase production of starch from domestic raw materials such as corn.

The diversion of cassava from the starch market would seem to lie in its fermentative utilization since the fermentation industries are the largest consumers of carbohydrate materials aside from the food industries. This would also be a step toward industrialization in tropical countries, such as the Philippines. Hence, studies, supported by a fellowship established by the University of the Philippines, were undertaken on the fermentative utilization of cassava.

Among the fermentation industries, production of butanol and acetone is surpassed only by the ethanol fermentation in amount of products formed. In this fermentation starchy materials are fermented anaerobically by *Clostridium acetobutylicum* with the production of butanol, acetone and ethanol, commonly designated as "solvents," in the approximate ratio of 60:30:10, respectively. The gases produced amount to about one and one-half times the weight of solvents formed, of which about 40 per cent is hydrogen and 60 per cent carbon dioxide, by volume. Industrially, the by-product gases are employed for the synthesis of methanol, and for dry ice production. In the commercial fermentation corn has been used largely as the raw material; recently blackstrap molasses has also become an important raw material for the production of the solvents mentioned, the process employing a different organism from that named above.

EXPERIMENTAL

The cassava samples were supplied by the College of Agriculture, University of the Philippines, Philippine Islands, and were obtained as dried chips from unpeeled roots; the chips were ground to a powder in a burr mill before use, the ground material being designated as *ground cassava.* The material analyzed 74.5 per cent starch. As far as is known ground cassava root has not been used in industry nor previously studied in the laboratory. It is the usual practice to peel the roots before use. Since peeling the roots is a rather laborious operation, the ground cassava represents a very much cheaper material than the peeled cassava.

The experimental mashes were prepared by mixing the requisite materials in 500-ml. Erlenmeyer flasks with water at 70° C. and gelatinizing the starch by heating. The mashes were then cooked in the autoclave at 20 lbs. of steam pressure for 40 min. They were cooled to incubation temperature and inoculated promptly with an active culture of the commercial butyl-acetone organism, *Clostridium acetobutylicum.* The methods of conducting the fermentations and of analysis were as outlined by Underkofler, Christensen and Fulmer (2). Correction was made for solvents from inoculum and other materials added, and the yields were calculated as per cent of glucose equivalent in the mash. The values given in the tables represent the averages for duplicate fermentations.

Corn is the standard substrate for fermentation by *Clostridium acetobutylicum.* A preliminary experiment to test the fermentability of cassava by this organism was conducted involving a systematic replacement of corn by ground cassava in such a manner that the total concentration of substrate was constant in all media. Each mash contained 18 g. of starchy material and 300 ml. of water. The weights of corn and cassava used are given in Table

Table I. Total Solvents Yields from Corn and Cassava Mixtures

Corn, % of total substrate	Corn, g. per flask	Ground cassava g. per flask	Total Solvents Yield, % of glucose equivalent
100	18.0	0.0	30.22
80	14.4	3.6	28.30
60	10.8	7.2	31.70
40	7.2	10.8	31.93
20	3.6	14.4	30.21
10	1.8	16.2	17.00
0	0.0	18.0	5.00

Glucose equivalent in corn: 76.5%; in cassava: 82.8%.

Table II. Total Solvents Yields from Cassava with Various Materials Added to Serve As Sources of Nutrients.

Amount of Nutrient Material Added, % of Cassava	Nutrient Material Used and Solvents Yield, % of glucose equivalent					
	Shrimp powder	Corn Gluten meal	Soybean flour	Compressed yeast	Peptone	Urea
0.0	1.9	1.9	1.5	1.5	3.9	3.9
0.5	9.6	3.9	2.5	2.5	5.2	4.9
1.0	13.7	3.9	6.5	4.9	10.0	6.1
2.0	22.1	13.1	11.4	7.0	14.7	6.4
3.0	27.6	19.1	18.2	7.0	18.2	6.6
4.0	30.2	23.1	21.2	12.7	19.3	7.8
5.0	27.6	29.2	25.6	16.2	23.5	9.7
Corn mash control	31.7	31.7	28.1	26.6	23.6	23.6

I along with the yields of solvents obtained from the fermentations. The experimental results showed clearly that cassava alone gave poor yields of solvents. However, as much as 80 per cent of the corn could be replaced by the ground cassava and still yields of solvents were obtained which were as good as from corn alone. It was therefore surmised that the poor fermentability of the cassava alone was due to deficiency of nutrients for the bacteria.

To test this assumption several materials which might serve to furnish nutrients were employed with ground cassava, and the resulting mashes were subjected to action of the butyl-acetone organism. Each flask contained 20 g. of ground cassava and 350 ml. of water. The amounts of the nutrient materials employed and the experimental results are shown in Table II. These experiments repeatedly confirmed that cassava by itself is a very poor substrate for the butyl-acetonic fermentation; the yields of total solvents were always low. The addition of small quantities of shrimp powder, corn-gluten meal, and soybean flour was found to increase the amount of total solvents to values comparable with those from corn. The optimum proportions of these materials was approximately 5 per cent of the weight of the cassava used. Compressed yeast and urea were not found suitable as nutrient sources. Peptone was excellent but its cost would be prohibitive on the industrial scale.

So far as is known shrimp powder has not been used previously in fermentations as a source of nutrients. The use of shrimp powder was considered especially promising because of its ready availability in the Philippines.

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