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The application of fermentative processes to the problem of the industrial utilization of agricultural products and by-products for the production of organic acids and 'solvents' has been approached from a number of angles. During the course of related studies the possibility of the fermentative utilization of artichoke tubers was considered. In many ways this plant would provide an agricultural product highly suited to such purposes. The artichoke is indigenous to North America and grows freely in all parts of the country. It can be machine cultivated in the same manner as corn and can be stored in the ground until needed (1). It is extremely hardy, adapts itself to great variations in soil and climate and is resistant to frost, while the foliage is very resistant to the ravages of insects and fungous diseases. Yields have been reported of from seven tons (5) up to twenty tons per acre (2).

In these experiments the artichoke tubers were prepared by grinding and drying the ground pulp at 80° C. The dry material remaining had the following composition:

Moisture	3.6	per cent
Pentosans	4.4	" "
Lignin	4.5	" "
Cellulose	3.8	" "
Ether extract	0.5	" "
Ash	4.7	" "
Proteins	7.6	" "
Pectin	11.1	" "
Total sugars	22.1	" "
Dextrins	36.5	" "

Methods used:

Pectin by method of Nanji, Paton and Ling (4)
Cellulose by method of Mehta (3)
Lignin by Shorger's method (8)
Pentosan by method of Reynolds, Osburn and Werkman (7)

The remaining constituents were determined as described in the Official Methods of the Association of American Agricultural Chemists. The sugars and dextrins are calculated as dextrose. The fraction reported as dextrins consists of those constituents not extracted by 90 percent but extracted by 10% ethyl alcohol. It probably consists of inulin and related levulosans.

The first experiments were undertaken to study the crude culture fermentation of artichokes under anaerobic conditions at thermophilic temperatures. The medium consisted of 100 gm. of the ground tubers in two liters of inorganic solution containing 0.1 percent $(NH_4)_2SO_4$, 0.1 percent K_2HPO_4, and 0.05 percent $MgSO_4$. Material for inoculation was obtained from manure compost, garden soil, stable manure and lake mud. Enrichment cultures were prepared by inoculating tubes of artichoke broth with these materials, incubating for 24 hours anaerobically and transferring. After two or three transfers, the culture was used to start the large fermentation. The fermentations were neutralized by periodic additions of sodium hydroxide or by growing in the presence of an excess of calcium carbonate or sodium bicarbonate acting as buffers.

The fermentations were under way in 24 to 48 hours with vigorous evolution of gas and were generally complete in six to eight days although when incubation was continued a very slow fermentation continued up to twelve or fourteen days as indicated by gas evolution. This slow fermentation is, in all probability, due to a bacterial attack on the more resistant portions of the plant residues. The chief products of fermentation were formic acid, acetic acid, carbon dioxide and hydrogen. The gas mixture contained from four to six times as much carbon dioxide as hydrogen on the molar basis. The acids consisted of a mixture of acetic and formic acids varying from 30 percent to 60 percent formic. In some cases relatively small quantities of propionic acid were found. This, however, never amounted to over 7 percent of the total. The weight of volatile acids averaged 15 percent by weight of the dried tubers.

Fermentations produced by inoculations from different sources showed remarkable uniformity. The variations in products were no greater than those between different fermentations produced by the same inoculum. In Table I are shown the results of acid production resulting from fermentations brought about by inoculations from three different sources.

Results shown in Table I indicate that the conditions maintained are favorable for the development of a particular type of bacterial

Table I — Acid production from artichoke tubers by crude cultures

SOURCE OF INOCULUM	CC. N. ACID PER 100 GMS. TUBERS	MOL. PER CENT ACIDS			GMS. ACID PER 100 GMS. TUBERS		
		Formic	Acetic	Propionic	Formic	Acetic	Propionic
Lake mud	260	38	57	5	4.55	8.9	0.96
Stable manure	236	44	49	7	4.77	6.94	1.6
Garden soil	172	35.1	60.1	5	2.76	8.25	0.64

flora which is common to the various sources used as inoculum.

Osburn, Stritar and Werkman (6) in similar studies on the fermentation of beet pulp found that repeated transfer of the crude culture resulted in an increased production of formic acid. With the same type of fermentation of artichoke tubers, similar treatment results in no pronounced change in acid production. In Table II are shown the results on acid production resulting from a crude culture carried through four transfers.

Table II. Effect of transfer of crude culture on acid production

Transfer	cc. N. acid per 100 gms. tubers	Acids—mol. per cent			Gms. acid per 100 gms. tubers		
		Formic	Acetic	Propionic	Formic	Acetic	Propionic
1st	220	51	47	2	5.21	6.8	0.3
2nd	177	30	67	3	2.4	7.0	0.4
3rd	159	20	75	5	1.5	7.2	0.6
4th	211	44	54	2	4.3	6.9	0.3

In general, higher yields of acids were obtained from those fermentations carried out in the presence of sodium bicarbonate as a buffer rather than with calcium carbonate. Attempts to change the acid ratio by carrying out the fermentation at different pH levels had no significant effect. At lower pH levels the fermentation was less complete while the acid ratio was not appreciably altered. From the average of a number of fermentations in the presence of sodium bicarbonate the calculated yield of acids would approximate 85 pounds of formic and 140 pounds of acetic per ton of tubers.

Attempts to bring about the fermentation of artichoke tubers by *Cl. acetobutylicum* with the production of butyl alcohol and acetone were uniformly unsuccessful. The fermentations were of brief duration producing only traces of solvents with acetic and butyric acids in a ratio of 70% of the former to 30% of the latter. Previous investigators, Nathan (5) and Thaysen and Green (9) have reported a similar failure in attempts to convert the carbohydrate of artichokes to 'solvents' by means of the butyl-alcohol organism. Thaysen and Green found that preliminary acid hydrolysis followed by suitable dilution rendered the carbohydrates available.

Further experiments were carried out to test the acid production from artichokes by the organisms of the genus *Propionibacterium*. Fermentation of the tubers by *Propionibacterium arabinosum* at 30° C. gave relatively high yields of volatile acids amounting to approximately 20 percent by weight of the tubers. The addition of considerable portions of nitrogen led to a marked increase in the acid production. Results of two such fermentations are shown in Table III.

Table III. Acid production from artichoke tubers by Propionibacterium arabinosum

ADDITION	TOTAL ACID CC. NORMAL PER 100 GMS. TUBERS	ACIDS — MOL. PER CENT			WEIGHT OF ACID PER 100 GRAMS TUBERS		
		Formic	Acetic	Propionic	Formic	Acetic	Propionic
None	324	3.23	32	64.5	0.48	6.52	15.16
150 grams corn gluten	558	2.0	36.4	61.7	0.51	12.2	25.45

Calculations on the basis of the fermentation of the artichokes with no extra addition of nitrogen show a yield of 302 pounds of propionic, 131 of acetic and 10 of formic acid per ton of tubers. With the addition of extra nitrogen in the form of corn gluten this yield rises to 508 pounds of propionic, 244 of acetic and 15 of formic per ton of tubers.

CONCLUSIONS

The anaerobic fermentation by crude cultures of artichoke tubers at thermophilic temperatures results in the production of acetic and formic acids with carbon dioxide and hydrogen as the only gasses. The carbohydrates of the artichoke tuber are not readily available to attack by *Clostridium acetobutylicum.*

Fermentation by *Propionibacterium arabinosum* gives relatively high yields of propionic and acetic acids which are further increased by the addition of nitrogen in the form of corn gluten.

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