ENCLOSURE (B) 2

RESEARCHES ON ALCOHOL FERMENTATION

(In Four Parts)

by

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Prepared for and Reviewed with Authors
by U. S. Naval Technical Mission to Japan

Research Period: 1944-1945

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ENCLOSURE (B)2

PART I

by

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Research Period: 1945

Ethanol fermentation of wood sugar produced by the Bergius method was studied. Production by Bergius method has not progressed far, since only 3 samples were studied. It was intended to continue this study, but time was not available.

Three samples were prepared in the following manner:

1. HCl in wood sugar neutralized with $BaCO_3$.
2. Ba in above mentioned sample was precipitated with H_3PO_4.
3. PO_4 in above sample was precipitated with $Ca(OH)_2$.

The sugar concentration of each sample was ca. 7%.

Rice bran (2%) and $(NH_4)_2SO_4$(0.7% based on total sugar) were used as a nitrogen source.

The percentage of produced alcohol in wood sugar mash was ca. 2.8-3.0%, and percentage of fermentation for theoretical value was ca. 72-78%.

The results of sample No.3 were most excellent, and sample No.2 was next. This data has not been kept because it was intended to repeat the experiment. No further data are available.

No commercial application of the Bergius process for obtaining alcohol from wood has been undertaken.

A photograph of the pilot plant used in fermentation tests is shown in Figure 1(B)2.

ENCLOSURE (B)2

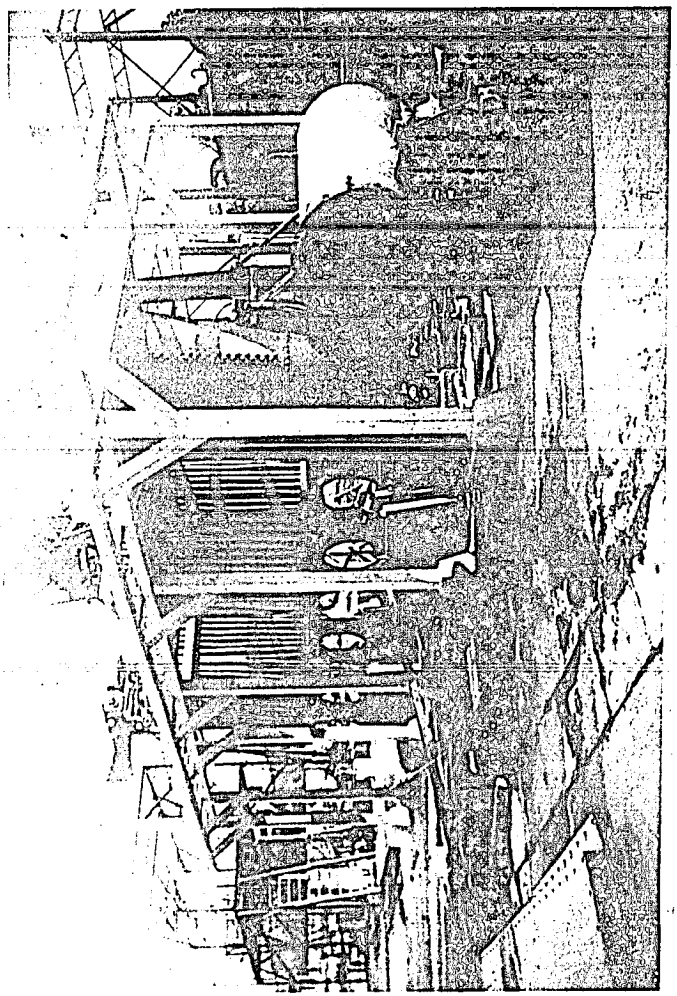

Figure 1(B)2
PILOT PLANT FOR FERMENTATION

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PART II

by

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Research Period: 1945

SUMMARY

Flask-tests were conducted with the object of determining whether or not corn and kaoliang can be used for raw materials of alcohol manufacture by Koji process without the use of a nitrogen source. The following significant results were obtained:

Corn showed good results (a) for the manufacture of Koji, (b) for saccharizing power of Koji and (c) as a raw material of fermentation.

The saccharizing power of unrefined kaoliang is inferior to that of corn, and it was not found to be a satisfactory raw material for fermentation; that is to say, the fermentation was slow and subject to putrefaction. However, it may be possible that kaoliang can be used for alcohol manufacture.

I. INTRODUCTION

A. History of Project

Because of the increasing importance of alcohol as an aviation fuel in Japan, it was deemed necessary to study whether or not corn and kaoliang could be used as the raw materials of alcohol manufacture. It was expected that a large amount of corn would be imported from the continent. The work was begun by investigating the fermentation by the Koji process.

B. Key Research Personnel Working on Project

Chemical Engineering Sub-Lieut. T. FUKUZUKO

II. DETAILED DESCRIPTION

A. Test Procedures

1. Mold and Yeast Used for Test

For Koji: Aspergirus Oryzae (manufactured at TANAKAYA in KYOTO).

Yeast: Toki-yeast.

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2. Method of Analysis

Acid Value: Reported as cc of 0.1 N NaOH for neutralization of 10cc of sample.

pH: pH test paper (manufactured by Toyo Filter Paper Co. Ltd.)

Saccharide: Fixed quantity determined by Lehn's method.

Alcohol (Ethanol): The alcohol content of the distillate of 100cc sample was determined.

3. Manufacture of Koji

Koji was prepared in a large Petri dish in a thermostat maintained at 33°C. The Koji were removed after 48 hours without any additional treatment.

4. Soaking Apportionment:

Yeast mash*	100cc
Koji	40gm
Raw materials	200gm
Water	1300cc

Note: Yeast mash: Mash of rice was saccharized previously by Koji of bran, 1% lactic acid was added and then pure culture yeast mash (about 10%) was added to this mash. The mixture was then fermented.

B. Test of Koji for Saccharizing Power

1. Preparation of Enzyme

Twenty grams of Koji were added to 200cc of water (35°C) and kept for 2 hours in a thermostat at 35°C. This enzyme solution was filtered. Ten cc of above enzyme solution were added to 50cc of 2% starch solution and then saccharized for 2 hours in a thermostat at 55°C. A portion of this saccharized liquid was used for analysis. The quantity of saccharide was analized by Lehn's method and was recorded as glucose.

2. Experimental Results

Table I(B)2 shows the results of the saccharizing power test, and Table II(B)2 shows the results of the analysis of the products of the fermentation of Tests No. 7 and No. 6.

III. CONCLUSIONS

A. As shown in Tables I(B)2 and II(B)2, corn has a rapid fermentation of about 80%. This indicates that corn is a good source of Koji.

B. In the case of kaoliang, the rate of fermentation is slow, acid value is high, and putrefaction occurs. Kaoliang does not appear to be a good source of Koji. This may be due to the effect of tannin which is included in kaoliang bran. If this is the case, it will be necessary to test refined kaoliang.

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G. Industrial application of the use of kaoliang as a raw material will necessitate the following studies:

1. The steaming method.
2. Development of a satisfactory crusher.
3. The effect of steaming and crushing on soaking time.
4. The kaoliang mash is very viscous and the sugar content cannot be concentrated by high pressure steaming. Some other method must be devised for concentrating the sugar.

Table I(B)2
SACCHARIZING POWER TEST IN ALCOHOL FERMENTATION

Test No.	Apportionment of Koji	Enzyme Solution			Glucose in 60cc Saccharized Liquid (gm)	Quantity of Glucose Saccharized from Starch (gm)	Total Sacch. quantity in the Starch Soln. 50cc (gm)	The Rate of Saccharizing power (%)
		Acid Value (cc)	pH	Glucose (gm/10cc)				
1	Bran 10: Chaff 5	1.45	6.4	0.0540	0.5568	0.5028	0.8796	57.2
2	Corn 5: Bran 3: Chaff 4	1.10	5.8	0.0627	0.5376	0.4743	0.8796	53.9
3	Corn 10: Chaff 1	1.15	5.4	0.1785	0.7046	0.5261	0.8796	59.3
4	Corn 10: Chaff 1	0.90	5.6	0.1126	0.6221	0.5095	0.8796	57.8
5	Only Corn (Sifted to 1 mm mash)	0.90	5.4	0.0874	0.7478	0.5604	0.8796	75.2
6	Kaoliang 10: Chaff 1	0.80	5.3	0.1220	0.6053	0.4833	0.8796	54.9
7	Kaoliang 10: Chaff 1	0.70	5.4	0.0927	05784	0.4857	0.8796	55.2

Table II(B)2
ANALYSIS OF THE PRODUCTS OF ALCOHOL FERMENTATION

Test No.		Before Fermentation			Time of Total Ferment (hr)	Volume (cc)	Acid Value	pH	Total Residual Glucose	Ethanol (Vol %)	Rate of Consumption of Saccharide(%)	Rate of Ferment. (%)
		Acid value	pH	Total glucose (%)								
4	Corn-Koji Corn-Mash	3.6	4.7	11.81	64	1400	5.4	4.4	1.076	6.0	90.0	79.0
6	Kaoliang-Koji Kaoliang-Mash	2.8	4.7	11.9	93	1450	11.0	3.6	1.213	4.7	89.4	61.4

Note: Total glucose before fermentation includes saccharides from yeast mash.(17.75%)

ENCLOSURE (B)2

P A R T III

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Research Period: 1945

SUMMARY

The conditions of ethanol fermentation when corn is digested at low pressures were investigated. The following significant results were obtained:

1. Corn was crushed to a 3mm-mesh powder, and digested for 7 hr under 1.75 kg/cm^2 pressure. When HF was added to this corn mash (ratio of HF to corn is 1/20000), the fermentation was 81% of the theoretical value.

2. Fine powder was not suitable for fermentation, since the pipes were clogged by the formation of dumplings which interfered with the fermentation.

3. The results shown in Table III(B)2 were obtained.

I. INTRODUCTION

A. History of Project

In Japan, cultures of aspergillus oryzae supply the diastase for the saccharification of grain starch in the manufacture of the drink known as koji made from rice, or take koji made from wheat bran.

Before saccharification, it is digested for sterilization and conversion of the starch in the grain by heating under pressure. When the corn is digested under low pressure, the conditions of ethanol fermentation are unsatisfactory. The purpose of this experiment was to investigate these conditions. In the original process, the procedure for the fermentation of grain was carried out in the following stages:

1. Conversion of the starch by heating under pressure.
2. Saccharification of the starch by diastatic action of the mould.
3. Fermentation of the sugar by yeast.

Figure 2(B)2 is a simplified flow sheet of the ethanol fermentation of corn.

B. Key Research Personnel Working on Project

Chem. Eng. Lt., Y. NODA

ENCLOSURE (B)2

II. DETAILED DESCRIPTION

A. The First Experiment

1. Material of Mash and its Analysis.

Corn.....quartered
Starch value.....61.24%
Directly reduced sugar.....4.27%

2. Condition of Digestion.

Time.....11 hrs
Pressure.....1.75kg/cm^2

3. Condition of Saccharification. Saccharification was performed with take koji (aspergillus oryzae), the temperature of saccharification was 55°C, and the time of saccharification was 2.5 hr.

4. Material of Yeast. Dried strips of sweet potatoes were used and the conditions were as follows:

Alcohol.....3.4%
Acidity.....5.45cc/10cc mash
pH.....4.2
Volatile acid.....0.1
Balling.....6.5

5. Material of Koji. The material composition was as follows:

Dried strips of sweet potatoes.....40%
Pupa oil cakes.....10%
Wheat bran.....20%
Rice hulls.....30%

Total.....100%

This koji material was cultivated with Aspergillus Oryzae.

6. The yield of alcohol was 45.8% of the theoretical and the results are tabulated in Table IV(B)2.

B. The Second Experiment

1. Material of Mash. The same corn as in previous experiment.

2. Conditions of Digestion.

Time.....7 hr
Pressure.....1.75 kg/cm^2

3. Condition of Digestion.

Temperature.....55°C
Time.....2 hr

4. Material of Yeast. The yeast was the same as in the previous experiment and was used under the following conditions.

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Alcohol.....2.4%
Acidity.....5.3cc/10cc mash
pH.....4.6
Volatile acid.....0.3
Balling.....4.6

5. Material of koji. Same as in previous experiment.

6. The yield of alcohol was 45.4% of the theoretical value and the results are tabulated Table V(B)2

C. The Third Experiment.

1. Material of mash. The same corn as in previous Experiment.

2. Condition of Digestion.

Time.....6 hr
Pressure.....1.75 kg/cm^2

3. Condition of Saccharification.

Temperature.....55°C
Time.....2.5 hr

4. Material of Yeast. It is the same as in previous experiment and was used under the following conditions:

Alcohol.....2.6%
Acidity.....4.7cc/10cc mash
pH.....4.4
Volatile acid.....0.15
Balling.....8.57

5. Material of koji. It is the same as mentioned above.

6. The yield of alcohol was 63.4% of the theoretical and the results are tabulated in Table VI(B)2

D. The Fourth Experiment.

1. Material of mash and condition of digestion were the same as mentioned above.

2. Condition of saccharification.

Temperature.....55°C
Time.....2.33 hr.

3. Material of Yeast. It is the same as mentioned above and was used under the following conditions:

Alcohol.....2.8%
Acidity.....4.9 cc/10cc
pH.....4.6
Volatile acid.....0.1
Balling.....7.77

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4. Material of Koji. Material components are the following:

Corn.....80%
Rice hulls.....20%

5. The Result. In this experiment, HF(40% aq. soln.) was added to the mash, and the ratio of HF/mash was about 1/20000. The yield of alcohol was 81.3% of theoretical and the results are tabulated in Table VII(B)2

I. CONCLUSIONS

ing HF in the fermentation, good yields of alcohol were obtained by the low ssure fermentation process.

Table III(B)2
SIGNIFICANT RESULTS OF CORN FERMENTATION AT LOW PRESSURES

(Pressure: 1.75kg/cm^2)

. of xp.	Degree of crush	Digested time, hr.	Added ratio of HF	Total sugar	Rested sugar	% of alcohol	% of fermentation
1	Quartered	11	0	11.69	4.59	3.5	45.8
2	Powder	7	0	12.38	3.55	3.6	45.4
3	Powder	7	0	10.05	2.13	4.1	63.4
4	Powder	7	1/2000	10.33	1.42	5.4	81.3

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Table IV(B)2
CORN FERMENTATION AT LOW PRESSURE

(Corn Was Quartered)

Time (hr)	pH	Acidity (cc)	Sugar (gm)	Temperature (°C)	Alcohol (%)
0	5.0	4.0	11.685	28	0
6	4.6	5.5	11.325	30	0.8
18	4.4	8.5	8.626	32	2.5
24	4.0	10.5	7.135	34.5	3.0
30	3.8	11.7	6.235	34.5	3.3
36	3.8	12.6	5.827	34.5	3.3
42	3.7	13.7	5.625	34.5	3.4
48	3.7	15.0	5.472	33	3.5
54	3.5	15.5	4.852	34	3.5
66	3.4	15.6	4.657	34	3.5
72	3.4	15.7	4.640	33	3.5
78	3.4	16.0	4.640	33	3.5
84	3.4	17.2	4.590	33	3.5

Table V(B)2
CORN (POWDER) FERMENTATION AT LOW PRESSURE

(Concentration of Mash: 12.38%)

Time (hr)	pH	Acidity (cc)	Sugar (gm)	Temperature (°C)	Alcohol (%)
0	5.2	2.8	12.38	29.5	0
6	4.8	4.5	10.21	31	0
12	4.6	5.5	7.34	35	2.9
18	4.6	6.9	7.08	36	3.0
24	4.4	8.6	6.73	36	3.5
30	4.4	10.4	5.46	36	3.5
36	4.4	12.0	5.23	36	3.5
42	4.2	13.0	4.97	35	3.5
48	4.2	13.3	4.03	35	3.6
54	3.8	14.0	4.02	35	3.6
60	3.7	14.8	3.97	34	3.6
66	3.6	15.4	3.94	34	3.6
72	3.6	15.4	3.92	34	3.6
78	3.2	15.6	3.62	34	3.6
84	3.4	16.4	3.55	34	3.6

ENCLOSURE (B)2

Table VI(B)2
CORN (POWDER) FERMENTATION AT LOW PRESSURE

(Concentration of Mash: 10%)

Time (hr)	pH	Acidity (cc)	Sugar (gm)	Temperature (°C)	Alcohol (%)
0	5.6	3.2	10.00	27.5	0
6	5.4	4.1	9.55	28.0	0.4
12	4.8	5.9	6.83	31.0	2.6
18	4.0	6.0	5.15	35	2.9
24	3.8	6.5	3.73	35	3.6
30	3.6	7.3	2.87	34.8	3.7
36	3.6	8.6	2.51	34.8	3.9
42	3.4	8.9	2.26	34.8	4.1
48	3.4	9.3	2.17	34.5	4.1
54	3.2	9.5	2.15	34.5	4.1
60	3.2	10.7	2.14	34.2	4.1
66	3.2	11.2	2.13	34.2	4.1

Table VII(B)2
EFFECT OF HF ON CORN FERMENTATION AT LOW PRESSURE

Time (hr)	pH	Acidity (cc)	Sugar (gm)	Temperature (°C)	Alcohol (%)
0	5.4	3.4	10.33	27.3	0
0	5.2	4.1	9.97	28	0.2
12	5.0	4.4	4.95	31	2.6
18	4.6	4.5	4.26	35	3.5
24	4.4	4.6	3.11	36	4.6
30	4.3	4.3	2.13	36	5.4
36	4.2	4.2	1.68	36	5.4
42	3.8	3.8	1.55	36	5.4
48	3.8	3.8	1.49	36	5.4
54	3.6	6.3	1.44	35.5	5.4
60	3.4	6.5	1.43	35	5.4
66	3.4	6.9	1.42	34.5	5.4

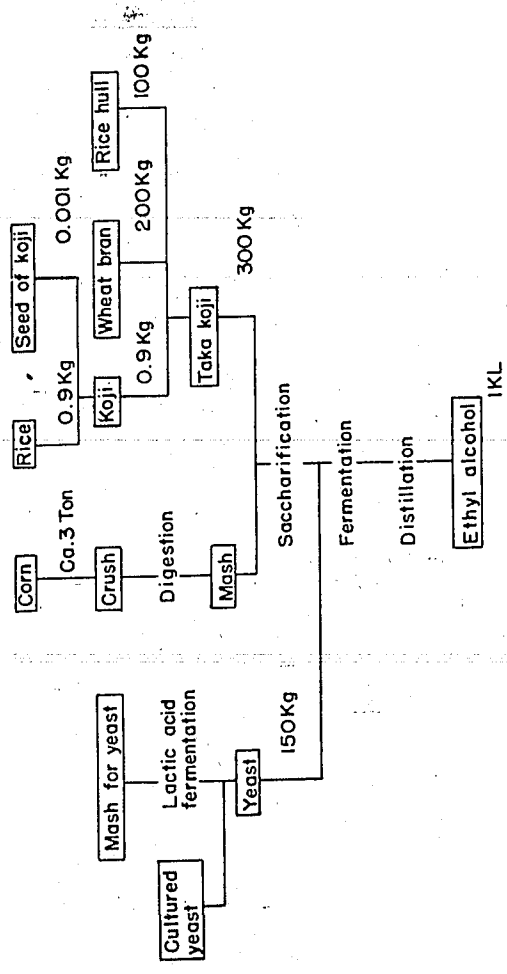

Figure 2 (B)2
FLOW SHEET OF THE ETHANOL FERMENTATION OF CORN
(This is not experimental data)

ENCLOSURE (B)2

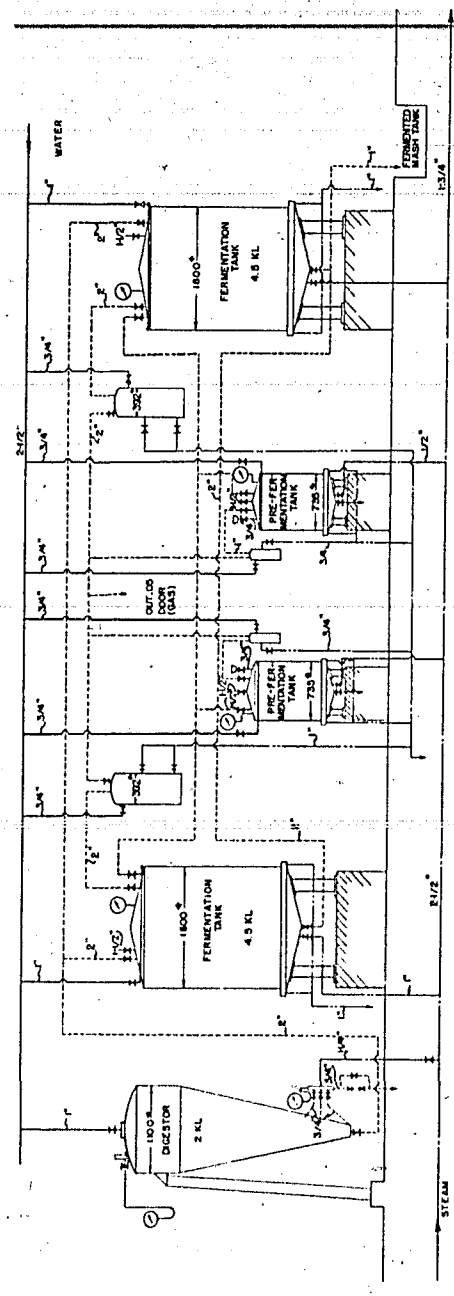

Figure 3 (B)2
FLOW SHEET OF FERMENTATION APPARATUS

ENCLOSURE (B) 2

P A R T IV

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Research Period: 1944-1945

SUMMARY

The most suitable raw materials for the koji in the alcohol fermentation of sweet potatoes was studied. The corn-koji showed a good result, and it's saccharifying power was nearly 93% that of the wheat-koji.

I. INTRODUCTION

In alcoholic fermentation, the sweet potato must be saccharified before being subjected to the action of the yeast. There are three saccharifying processes used:

1. The Amyromyces method
2. The mineral acid method
3. The koji method

In this project the koji method was used.

There are two materials used in the Aspergillus group for saccharifying sweet potatoes; namely, aspergillus oryzae, the so-called "White Koji", and Aspergillus awamori, the so-called "Black Koji".

However, the raw materials in the culture of moulds have a great influence on the saccharifying power of the mould. The most suitable raw material for these moulds, from an economical point of view, has previously been rice bran. In this research, raw materials other than rice bran were used. In the case of Aspergillus oryzae or "White Koji", kaoling with rice bran, corn with rice bran, were selected as suitable raw materials for growth of the moulds.

The cultures of the mould of with these raw materials was not perfect since the power of saccharification was only 93% of the yield using rice bran. Aspergillus awamori (Black Koji) showed comparable results.

It is assumed that the culture superior in saccharifying power will give equally good results in fermentation experiments. Therefore, the authors studied the saccharifying power of various raw materials.

II. DETAILED DESCRIPTION

A. Details of Test Procedures and Conditions

1. The raw materials mentioned above were steebed for 14 hr, and then boiled for 50 min. After this treatment the spores of the mould were mixed with these materials, and they were kept at 28°C,

for 48 hr in a thermostat. During this process the raw materials were stirred frequently to maintain an equal temperature.

2. Experimental method of determining the saccharifying power of koji

a. Enzyme solution:-Twenty grams of koji were added to 200cc water, kept for 2 hr in a thermostat at 35°C, and filtered.

b. Starch solution:-A 2% soluble starch solution was prepared.

The enzyme solution (50cc) and starch solution 910cc) were mixed in a flask, and kept for 2 hr at 55°C.

The quantity of reducing sugar after saccharification was determined and it was expressed as the degree of saccharification.

3. Method of Analysis

Sugar was determined by Hanes' method and calculated as glucose.

The pH value was determined by test paper specially made for the purpose by the Toyo Filter Paper Co, Ltd.

B. Experimental Results:

1. The results of experiments on the saccharifying power of Asp. Oryzae prepared from different raw materials are shown in Table VIII(B)2.

2. The results of the experiment on the saccharifying power of Asp. awamori prepared from different raw materials are shown in Table IX(B)2.

3. Experiment on the fermentation of sweet potato mash by koji.

Asp. oryzae was found to be more powerful than the Asp. awamori in saccharifying power as determined above. Hence, in this experiment, Asp. oryzae was used in the cultivation of rice or a mixture of corn and rice bran.

a. Anglyis of Sweet Potato. Name of the variety is Genji.

Water	70.1%
Crude fiber	1.0%
Total N.	0.1%
Starch value	24%

b. Preparation of Mash

A 385 gm sample of sweet potatoes was placed in a flask, and a small amount of water added. The mixture was ground slightly and was sterilized in the usual manner.

c. Yeast

A 10cc sample of koji extract (balling-10) was placed in a test tube, and sterilized by the usual method.

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Yeast was innoculated to this medium and incubated for 24 hr at 30°C.

d. Saccharifying and Innoculation

Ten percent by weight of koji was added to the sweet potato in the flask and the mixture was incubated at 55°C with frequent shaking. After this treatment, the mash was cooled to 30°C, and the yeast was innoculated.

e. Method of Analysis

The pH and acidity were determined as usual. Total sugar was determined by Hanes' method. The alcohol in the distillate of the fermented mash was determined by specific gravity.

f. These results are shown in Table and Table XI(B)2.

III. CONCLUSIONS

1. It was recognized that the saccharifying power of Asp. Oryzae was greater than that of Asp. awamori.

2. The corn koji was shown to have a good saccharifying power, and it was nearly 93% that of the rice bran koji.

3. Other materials such as, kaoliang (1) plus corn (5), and a mixture of castor seed cakes, corn, and rice hulls, are also useful raw materials for the koji.

4. Experiment proved that other types of koji besides rice koji are capable of application in industry.

ENCLOSURE (B)2

Table VIII(B)2
DETERMINATION OF THE SACCHARIFYING POWER OF ASP. ORYZAE

Test No.	Raw materials	pH Before Extract	After Extract	Before Saccharifying	After Saccharifying	Percent of Saccharification	Order of Saccharifying power
1	Rice	5.7	5.8	6.0	5.7	52	7
2	Rice bran	5.2	5.2	5.4	5.4	83	1
3	Vine of sweet potato	4.0	4.0	4.2	4.2	36	10
4	Corn 5: Rice hulls 1	4.4	4.4	5.0	5.0	77	2
5	Kaoliang 5 Rice hulls 1	4.8	4.8	5.6	5.4	74	4
6	Kaoliang	5.0	5.0	5.2	5.4	59	5
7	Corn 6 Castor seed oil Rice hulls 3	5.4	5.4	5.5	5.6	75	3
8	Corn 6 Castor seed cakes 1 Rice hulls 3	5.6	5.6	6.3	6.3	36	10
9	Cotton seed cakes 4 Vine of sweet potato 1 Castor seed cakes 1	5.0	5.0	4.8	4.8	40	9
10	Pupa seed cakes	4.2	4.2	4.5	4.5	36	10
11	Soya bean cakes	4.3	4.4	4.4	4.4	54	6
12	Rice bran (red)	4.0	4.0	4.2	4.2	47	8

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Table IX(B)2
DETERMINATION OF THE SACCHARIFYING POWER OF ASP. AWAMORI

Test No.	Raw materials	pH				Percent of Saccharification	Order of Saccharifying Power
		Before extract	After extract	Before Saccharifying	After Saccharifying		
1	Rice	4.7	3.4	5.4	4.8	58	2
2	Vine of sweet potato	5.4	4.7	5.4	5.4	39	5
3	Corn	3.0	3.0	5.8	4.8	66	1
4	Kaoliang 5 Rice hulls 1	2.8	2.8	5.8	4.8	33	6
5	Kaoliang	4.4	3.8	5.0	5.0	31	7
6	Corn 6 Castor seed cakes 1 Rice hulls 3	5.2	4.6	5.0	5.0	52	3
7	Soya bean cakes 6 Rice hulls 3 Castor seed cakes 1	5.4	5.4	5.4	5.4	45	4
8	Cotton seed cakes 1 Vine of sweet potato 1	5.4	5.4	5.4	5.4	11	8

ENCLOSURE (B)2

Table X(B)2
EXPERIMENT ON THE FERMENTATIONS OF SWEET POTATOES

Test No.	After Fermentation				Ratio of Fermentation	Ratio of the Consumed Sugar Based on Total Sugar
	pH	Acidity cc	Rested Sugar gm/100cc	Alcohol %		
1	4.0	4.7	1.0	7.9	.97	.93
2	4.0	5.3	2.0	7.4	.84	.86
3	4.3	3.1	4.0	6.0	.74	.71
4	4.2	4.6	1.6	7.5	.92	.82

Note: Koji material is rice.
Before fermentation pH is 4.1, acidity is 3.2cc, and total sugar is 14.3 gm/100cc.

Table XI(B)2
EXPERIMENT ON THE FERMENTATION OF SWEET POTATOES

Test No.	After Fermentation				Ratio of Fermentation %	Ratio of the Consumed Sugar Based on Total Sugar %
	pH	Acidity cc	Rested Sugar gm/100cc	Alcohol %		
1	4.1	4.1	0.65	7.7	87.5	95
2	4.0	4.3	0.64	8.5	96	98
3	4.2	4.5	0.5	8.0	91	97
4	4.1	4.0	0.5	7.9	90	97

Note: Koji material: Corn 5: Rice hulls 1
Before Fermentation, pH is 5.0, acidity is 1.9cc, and total sugar is 15.5 gm/100cc