ON THE DETECTION OF METHYL ALCOHOL IN ALCOHOLIC BEVERAGES AND ITS FORMATION BY THE SEVERAL KINDS OF YEASTS.

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One of us has already published a paper on the detection of methyl alcohol.[1] In that case, the beverages examined were "saké," shochiu (spirit of saké—mash—cake), a Scotch whiskey, cognac, a wine purchased in the market and another prepared in our laboratory, using a modification of Denigè's colorimetric method. Therefore, it is unnecessary to test for methyl alcohol in alcoholic beverages by the same method.

Recently, Yanagisawa and Maruyama have published a method for the detection of formaldehyde in "saké," based on Eury's method.[2] I quote from them:

"To 100 cc. of "saké" add 2 cc. of diluted sulfuric acid and distill. Lay aside the first distillate of 30 cc., and collect the next 20 cc., which is used for the formaldehyde test. To 5 cc. of the distillate in a tall beaker, add 5 cc. of an egg-white solution[3] and 15 cc. of hydrochloric acid,[4] then heat to the boiling point. After that compare the violet color produced with that of the controls; one in treating water and the other the solution of formaldehyde in 1:1,000,000 in the same way as the distillate obtained."

The method mentioned above induced us to observe again methyl alcohol in alcoholic beverages, but we distilled the beverage always below 80°, using a fractional column for the sake of getting the most suitable distillate for the purpose. After distillation, we have very safely oxidized by using potassium permanganate and sulfuric acid, as mentioned in Aweng's[5] or our official method.[6] In the distillation of the oxidized alcohol, the first part (acetaldehyde) was set aside by the test of Rimini's or Jean's reagent,[7] and after adding calcium carbonate, redistilling the distillate, the test was made on the last distillate thus obtained.

As a subordinate test for methyl alcohol, we applied Manzoff's[8] nitromethane method, and Rimini's test was not neglected for the test of the aldehyde obtained by the oxidation of the sample.

In carrying out the experiment, the samples were always made up to a volume of 200 cc., except for beer, in which case it was impossible to get a distillate below 80°, with a fractionating column, so that we took 300 cc. of the beer and after an ordinary distillation, the fractionating column

[1] Teizo Takahashi, *J. Coll. Agri. Imp. Univ. Tokyo*, **5**, 302 (1915).

[2] *J. of Pharm. Tokyo*, No. 412 (1916).

[3] A mixture of 1 part of an egg-white and 4 parts of the water.

[4] A litre of HCl contain 1 cc. of 5% solution of $Fe_2Cl_6 + 12H_2O$.

[5] E. Aweng, *Apoth. Ztg.*, **27**, 159 (1912).

[6] A modification of Aweng's method, cf. *J. Coll. Agri. Imp. Univ. Tokyo*, **5**, 302 (1915).

[7] *Apoth. Ztg.*, **27**, 159 (1912).

[8] *Z. Nahr. Genussm.*, S, 469 (1914).

was used for redistilling. In the case of the spirit, we diluted the sample until it contained about 20% of alcohol. The other details in regard to the oxidation of the distillate are just the same as our official method, quoted above.

The results are tabulated below:

(— denotes a trace, and + a weak, ++ a medium, and +++ a strong reaction)

The substance examined.	Yanagisawa's reaction.	Rimini's reaction.
(1) Wine purchased in the market:		
(*a*) Mont Ferland	+ dark violet	+
(*b*) Soint Sulpice	+ + " "	+ +
(*c*) Daikokuten (white)	+	+
(2) Whiskey purchased in the market:		
(*a*) The House of Commons	+ + +	+ +
(*b*) Glenlivet whiskey (Watson Co.)	+	+
(3) Saké:		
(*a*) M	—?	—
(*b*) C	—?	—
(*c*) G	+ reddish violet	+trace[1]
(*d*) S	—?	—
(*e*) C. M	—?	—
(*f*) S. G	—?	—
(*g*) S. T	+?	—?
(*h*) T	+?	—
(*i*) Institute of Saké Brewing	+ +	+ +
(4) Spirit:		
(*a*) Dainiho Pharmacet. Co	+ +	+ +
(*b*) From 300 cc. of the sample, four fractions were obtained by the distillation, and the examinations were made on each fraction:		
1 Fraction I (50 cc.)	+ + + +	+ + + +
2 " III "	+	+
3 " IV "	+	+ +
(*c*) To one pound of sample (*b*) lime was added and after two days, six fractions were obtained by distillation.		
4 Fraction VI	+	+
(*d*) The spirit sealed in bottle by our government sanitary experiment station	+	+ trace
(5) Beer:		
(*a*) Stout (imported from England)	—	—
(*b*) Y. B	+ trace	+ trace
(*c*) S. P	+	+
(*d*) S. B	+	+ trace

[1] In this sample, there was evidence of methyllactate, which is proved by anisaldehyde and sulfuric acid: cf. Teizo Takahashi, *Bull. Coll. Agri. Imp. Univ. Tokyo*, **7**, No. 4.

The substance examined.	Yanagisawa's reaction.	Rimini's reaction.
(*e*) K. R.	+?	+
(*f*) S. K.	+?	—
(6) Saké, manufactured at our Brewing Institute at Oji:		
(*a*) In the market	+ + +	+ + +
(*b*) No. 144	+	—
(*c*) No. 199	+	—?
(*d*) No. 138	+ +	+ +
(*e*) No. 149	+ +	+ +

[In all cases, the distillates were tested before the oxidation for the presence of formaldehyde, which was always absent.]

From the table we can easily ascertain that saké, in general, contains a smaller quantity of methyl alcohol than the other alcoholic beverages; the six samples brewed at our Institute of Brewing, and one signed G in the above table, being the exceptions in this regard. On the other hand, the distillate of beer, wine and spirit after oxidation gave marked Yanagisawa's reaction, although there was one exception.

Manzoff's nitromethane method, which we used as a confirmatory test, gave a positive proof of the presence of methyl alcohol in the spirit which we have tested.

We made some further examination of saké, which proved to be almost free from methyl alcohol when Yanagisawa's method was applied. For this purpose we took some larger quantities of "saké" than before *i. e.*, we have condensed methyl alcohol by the redistillation of the first distillate. The results are tabulated below:

Name of "Saké."	G. K.	S.	S. K.	K.	C. H.
Sample taken	Ca 700 cc.	do.	do.	do.	Ca 500 cc.
First distillate	80–90 cc.	do.	do.	do.	60 cc.
Distillate after addition of lime	Ca 20 cc.	do.	do.	do.	Ca 15 cc.
Nitromethane method	++	++	+	++	+
Yanagisawa's method	++	++	+	+	+

Thus we see that the existence of methyl alcohol was proved in "saké" by both Manzoff's nitromethane method and Yanagisawa's, if the sample taken is large.

A further investigation was made as to whether the varieties of the yeast or the pabulum in which the organisms were cultivated permitted the formation of methyl alcohol in alcoholic fermentation. The yeasts used in the research were beer-, "saké"-, and distillery-yeast in pure cultures. In the first series, "koji" extract was used as a nourishing medium.

The innoculation of the yeast was made after the ordinary treatment of the pabulum for sterilization, and the cultures were maintained at 25–30° during 12 days. After the end of the fermentation, the whole fluid was distilled and redistilled. The test for methyl alcohol was carried on in the second distillate, *i. e.*, a concentrated sample. The results of the analysis are as follows:

Yeast.	Pabulum.	Detection of methyl alcohol in media. Nitromethane method.	Yanagisawa's method after oxidation.	Rimini's method after oxidation.
"Saké" yeast A. No. 34 ...	"Koji"-extract	+	+	—
Wine yeast (Oppenheimer).	"	+ trace	+?	—
Beer yeast (Bottom).....	"	+	++	+?
Distillery yeast.....	"	+	+	—
Wine yeast (Oppenheimer).	Raisin extract	+	+	—?
"Saké" yeast A. No. 34....	Hayduck's solution	+	+(+)	—
do.	(1% glycocoll instead of asparagin in Hayduck's solution).	+	++	+?
Beer yeast.....	do.	+	+	+
Beer yeast.....	Hayduck's solution	+	++	+
Distillery yeast.....	" "	+	++	—

Thus, the formation of methyl alcohol by all kinds of yeasts examined is proved. Especially in the case of "saké" yeast, the quantity of methyl alcohol formed was increased when glycocoll was used instead of asparagin in Hayduck's solution. The other interesting fact to be noticed is the behavior of the wine yeast to be pabulum; the quantity of methyl alcohol formed was decidedly larger in the raisin extract than in "koji" extract, which, after the fermentation, was proved to be almost free of it.

Summary.

1. In the distillates of alcoholic beverages, which we have examined, we could not find formaldehyde directly when we distilled below 80°, but after the oxidation of the distillates there were found differences in regard to the quantities of methyl alcohol according to the kinds of alcoholic beverages. The smallest quantity was found in the case of "sake," except a special sample, in which there was a distinct evidence of methyl lactate.

2. In the case of a doubtful test a large sample was taken for analysis, followed by the redistillation of the distillate.

3. All the kinds of yeast tested, "saké-", beer-, wine-, and distillery-yeasts formed methyl alcohol in the saccharine fluids and the quantity of it is increased by the addition of glycocoll as a nourishment. This fact exactly coincides with Ehrlich's[1] theory of the fermentation of protein matter.

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ACCURATE METHODS FOR THE DETERMINATION OF TOTAL ACIDITY OF URINE.

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The purposes of this paper are (1) to show the influences of urea and

[1] Ehrlich, *Brewer's J.* (New York), 38, 144 (1914).