M. P.	Subst. g.	N.c.c.	P.m.m.	T	N. found	Calculated for
1. 122°	0.1183	22.7	759.2	25°	21.43%	21.76 (C ₉ H ₁₁ N ₃ O ₂)
2. 88-90	0.1075	19.9	755.7	25	20.57	20.30 (C ₁₀ H ₁₃ N ₃ O ₂)
3. 126	0.0526	9.7	759.0	25	20.59	20.30 (C ₁₀ H ₁₃ N ₃ O ₂)
4. 110	0.0846	14.4	758.7	23	19.20	19.01 (C ₁₁ H ₁₅ N ₃ O ₂)
5. 149	0.1008	18.8	764.8	18	21.68	21.76 (C ₉ H ₁₁ N ₃ O ₂)

III. Acetic acid formation from ethyl-alcohol by saké yeast.

Medium: ethyl-alcohol 240c.c. (alc. 96.2% ald. 0.002074%) water
2820c.c. yeast mud 57g.

Aldehyde and total acid after 66 days at 25°C was each 0.02038g. and 0.048g. per 100c.c. of culture.

Silver content of Ag-salt prepared from the distillate :-

Subst.	AgCl	Ag. found	Ag. calculated for C ₂ H ₃ O ₂ Ag.
0.2062g.	0.1748g.	63.78%	64.64%

IV. Oxidation of propyl alcohol by Bac. xylinum at 20°C.

Medium: a. propyl alcohol 30c.c., (NH₄)₂HPO₄ 0.5g. KCl 0.1g
water 970c.c.

b. a + CaSO₃ 20g.

Microbes: Bacteria-mud developed in 1 L. of koji extract.

(a.) Total acid after 35 days 0.2738g. per. 100c.c.

Silver content of Ag-salt prepared from the distillate.

Subst.	AgCl	Ag found	Ag calculated for C ₃ H ₅ O ₂ Ag
0.3282g.	0.2578g.	59.11%	56.62%

(b.) Aldehyde after 32 days 0.07948g. per 100c.c.

Character of p-nitrophenylhydrazone prepared.

M.P.	Subst.	N	N. found	N. Calc. for C ₉ H ₁₁ N ₃ O ₂
1210	0.1068g	20.9c.c. (25°C 756.2 mm)	21 76%	21.76%.

ON THE PRODUCTION OF ACETOIN AND 2,3-BUTYLENGLYCOL BY MICROBES AND THEIR DISTRIBUTION IN FERMENTATION PRODUCTS.

By MASAKAZU YAMADA and KANROKU KURONO.

(Received Apr. 22nd., 1927.)

It has often been reported that the reducing substance in vinegars is

acetyl methyl-carbinol.⁽¹⁾

On the other hand, the production of acetoïn and its related compound, 2,3- butylenglycol by microbes, especially by several bacteria has repeatedly been described under the name of butylen glycol-fermentation in the culture media containing glucose, fructose, mannit, glycerine or Ca-lactate as a carbon source.⁽²⁾ As to the production of both compounds by yeast Kluyver, Donker and Visser't Hooft confirmed that the glycol was formed everytime while acetoïn rarely in the sugar solution.⁽³⁾

The mechanism of their production is not yet accurately known except the explanation put forward by C. Neuberg and their associates. They say that acetaldehyde which is formed in nascent state as an intermediate product in the fermentation of the sugar undergoes an acyloïn like conjugation with acetaldehyde, when the latter is added to the fermented liquid, by aid of carboligase and the acetoïn thus formed, is reduced into butylen-glycol phytochemically.⁽⁴⁾ But Elion obtained acetoïn from the dilute ethyl-alcohol or acetaldehyde solution by the yeast.⁽⁵⁾

Lately T. Taira reported on the distribution of the butylenglycol in Japanese fermentation products, without referring to acetoïn. Therefore the production of these two compounds by microbes and their distribution in main fermentation products have been newly tested.

The results are as follows :-

1. Bacteria produced a considerable quantity of both compounds.
2. Yeasts and a fungus produced a little of both except *Pichia* and *Chalara*.
3. The butylenglycol existed in saké(fresh and old), putrid saké, shōyu, tamari-shōyu, wine, beer and vinegar, that is to say, in almost all kinds of fermentation products; it may be regarded therefore as an ordinary component of all fermentation products.
4. Acetoïn was found in putrid saké, shōyu, tamari-shōyu and vinegar.
5. Acetoïn may be taken as a component which distinguishes the imitated vinegar from the fermented one, for it was not detected in the former.
6. Also the detection of acetoïn may be used to distinguish saké and putrid saké while it must be careful that a minute quantity was found in

(1) C. A. Browne :

J. Pastureau :

Farnsteiner :

(2) A. Harden. u. G. S. Walpole : Proc. Roy. Soc. B. 77, 399, 1906; Harden u. Norris: Ibid. 84, 492. Ruot : C. r. Acad. 157, 247-99, 1913; M. Lemoigne : Ibid. 155, 792-95; 157, 653-55; 177, 652-54; C-r. Soc. Biol. 82, 984, 83; 336-8; 88, 467.

(3) Bioch. Z. 161, 361, 1925.

(4) C. Neuberg u. E. Rein forth : Bioch. Z. 143, 553, 1923.

(5) Elion : Ibid. 40-44,

"moto" - culture of saké yeast in saké brewing and koji extract culture fermented by saké yeast.

7. There was no production of both compounds in the dilute alcohol solution cultivated with saké yeast or acetic acid bacteria contrary to Elion's experiment.

Experimental

Medium : for yeast and fungus; 50c.c. of koji extract (10°Balling) for bacteria; 50c.c. of neutralized koji extract (10°B) to which 1.5g. of CaCO_3 is added.

Microbes : Each 1 platinum ear was inoculated.

Detection : By Lemoigne's method developed by Kluyver and his associates. (3) (Formation of Characteristic red crystal of Nickel dimethylglyoxim.)

Production or existence is shown in the following table.

O was not tested.

Microbes	Sample	Acetoïn	2,3-butylen-glycol	age of culture
B.lactis 1.	15c.c.	++++	++	12 days
2.	"	++	—	12
B.butyricus Hüppe	"	++++	++++	12
B.subtilis	"	++++	++++	12
B.pyocyaneus	"	++++	++++	13
B.mesentericus	"	+++	++	13
B.coli communis	"	++++	++++	13
B.proteus Vulgaris	"	++++	++++	13
B.acetosum xylinum Brown	"	++++	++++	13
" (glucose solution)	"	+++	++	13
" (3% alcohol Sol.)	"	—	0	13
Zygosacch. soja A	"	+	++	10
Schizosacch. pombe A	"	++	++	11
Pichia IV	"	—	—	11
Oidium A	"	++	++++	11
Mucor mucedo	"	++	+++	11
Chalara mycoderma	"	—	+	11
Monilia candida	"	++	++++	11
Mycoderma A	"	+	++	12
Willia anomala var saké I	"	+	++	12
koji extract (control)	"	—	—	0

Subst.	Vol	extractive agent	Acetoïn	Butylen-glycol
saké (fresh)	15c.c.	—	—	++
" "	650	ether	—	0
" "	"	CHCl_3	—	0
" (old)	"	ether	—	++

Shōyu	150	CHCl ₃	++	++++
Tamari-shōyu	100	CHCl ₃	++	++
wine	15	—	0	++
beer (Yebisu)	500	CHCl ₃	—	+
" "	15	—	—	—
vinegar	15	—	++	+++
moto	15	—	—	+
"	900	CHCl ₃	+	0
putrid saké	15	—	+	++
Koji ex. fermented by saké yeast	15	—	—	±
after 6 days				
"	500	CHCl ₃	++	+
Koji extract(not fermented)	2000	ether	—	—
5% alcohol solution	1400	ether	—	—
with saké yeast				