The Sunflavor of Beer. II. Essential Substance of Sunflavor.

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As reported in the previous paper[1] we have ascertained that the essential substance of sunflavor of beer is thioglycolic acid ethylester, and we could also prove chemically and biologically that the essential substance was produced from dithiodiglycolic acid diethylester (-S-S-) which originated in cystine within malt juice by the reducing function of certain unknown components in beer under exposure to sunlight.

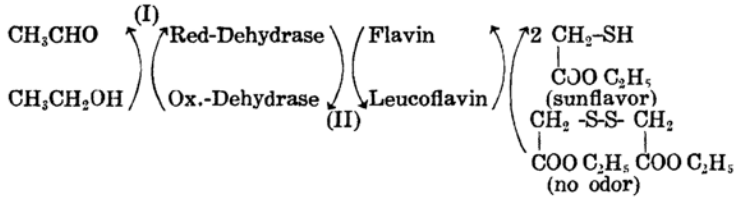
It has already been recognized that the malt of the raw material of beer contains the ferments belonging to dehydrase[2] and flavine[3] and that the solution of autolysed beer-yeast also contains a large amount of dehydrase[4] and flavin[5]. So we can easily expect that at least these

(1) Y. Obata and T. Yamanishi, *Agriculture*, (Japan) **9** (1947), 537.
(2) A. Foder and L. Frankenthal, *Biochem. Z.*, **225** (1930), 417.
(3) P. Karer and K. Schöpp, *Helv. Chem. Acta.*, **17** (1934), 1013.
(4) H. v. Euler and H. Adler, *Z. prakt. Chem.*, **235** (1935), 122.
(5) O. Warburg and W. Christian, *Biochem. Z.*, **266** (1933), 377.

ferments in beer yeast will be excreted into beer while its fermentation proceeds.

In fact it is reported that beer contains about 0.26 mg. of flavin per liter[6], and we could prove the existence of dehydrase in un-Pasteurized beer by the fact that such beer discolored methylene blue solution.

As we could presume that the flavin and dehydrase would take part in the development of the sunflavor of beer, we carried out an experimental study on the mechanism of sunflavor production with dehydrase prepared from beer yeast as well as with flavin. The result was just as we had presumed. Namely, we could prove that by the joint function of these two factors -S-S- was reduced and consequently sun-flavor was produced. This mechanism can be shown with chemical formula as follows:



The change of flavin to leucoflavin by dehydrase is accelerated by sunbeam.

Experimental Part.

Preparation of dehydrase solution. (i) Preparation of dehydrase acetone-sample. Concentrated suspension of beer-yeast which was previously washed twice with water, is cooled with ice, to which is added cold acetone gradually while stirring vigorously. By this treatment the cell membranes of beer-yeast are broken, and the suspension becomes voluminous.

Then it is rapidly filtered by suction, washed twice with acetone and finally washed once more with ether. By drying the residue in vacuum desiccator for one day white powder is obtained.

(ii) Preparation of dehydrase solution. 5 g. of dehydrase-acetone sample thus prepared is suspended in 100 c.c. of K_3PO_4 solution (*p*H 7.2) and kept in the thermostat at 28° C for two hours. When it is filtered, a clear pale yellow colored solution is obtained.

Experiment on the sunflavor production. (1) By dissolving K_3PO_4 in 3% alcohol, the latter is made to have *p*H 7.2 (optimum *p*H of alcohol-dehydrase). Two colorless glass vessels with a glass-stopper containing 10 c.c. are filed with the solution composed of 8 c.c. of the above mentioned alcohol, 2 c.c. of dehydrase solution and 1 mg. of flavin (Sankyo's injection drug).

(6) S. Komatsu et. al., *Chem. and Eng.*, (Japan) **41** (1938), 158.

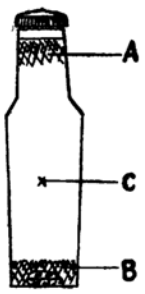
One bottle is kept in darkness and the other is exposed to sunlight.

After 90 min., the content of the former shows no change, while that of the latter becomes completely colorless and tells the change of flavin to leuco-flavin.

If one drop of -S-S- is sunk in this colorless liquid, the yellowish color comes back around -S-S-.

This means flavin is changed to the oxidized form. Accompanied with this change sunflavor is clearly produced.

If the solution used in this experiment lacks 3% alcohol or keeps its acidity at *p*H 5.4, its discoloration after 90 min. exposure to sunlight will be very faint.



A: Yellow, oxidation by air.
B: Yellow, oxidation by -S-S-.
C: Colorless.

Thus it can be concluded that in the reaction-system under consideration alcohol is necessary as its substrate and the optimum *p*H is at 7.2. These conditions coincide with those concluded in the previous report of one study connected with the sunflavor production by beer-yeast.

(2) On examining if dehydrase or flavin added separately can generate the sunflavor, it was concluded that their joint addition was necessary to induce the phenomenon in question as shown in Table 1.

Table 1.

Condition	Reaction system and sunflavor production	
Exposed in sunlight	-S-S- (−)	-S-S-+D (±)
,,	-S-S-+F (±)	-S-S-+D+F(*) (+)
Kept in darkness	-S-S-+F (−)	-S-S-+D+F (−)

(+) and (−) denote respectively that sunflavor is generated or not generated after 90 min. -S-S- denotes the substrate solution of the reaction which is at *p*H 7.2 being composed of 100 c.c. of the saturated solution of dithiodiglycolic acid diethylester, 3 c.c. of alcohol and 2.5 gr. of K_3PO_4. F denotes 2 mg. flavin (injection drug) dissolved in 20 c.c. of K_3PO_4 solution (*p*H 7.2). D denotes dehydrase solution prepared from beer yeast as in the experiment (1).

These three kinds of solution are mixed in the following proportion. One c.c. each of D and F are taken and poured together in the glass vessel explained in the experiment (1).

Then the remaining space of the vessel is filled up with -S-S-.

As the vessel is thus filled up with reaction solution perfectly, the experiment is carried out under an anaerobic condition.

(3) Test on the presence of dehydrase in beer and destruction of dehydrase by heating.

The glass vessel explained above was filled with 8 c.c. of un-Pasteurized beer and 2 c.c. of $^1/_{25000}$ methylene blue solution, and kept at room temperature.

Even after 3 days the color of the solution showed little change.

To accelerate the reaction *p*H of un-Pasteurized beer was raised from its natural value 4.2 to the optimum for the function of dehydrase or 7.2 and the vessel was placed in the thermostat regulated at 22°C. The color of the solution then faded perfectly after 2 days.

Un-Pasteurized beer comes very quickly to give off sunflavor while Pasteurized beer does so very slowly. This fact suggests that the dehydrase becomes functionless by heating. Pasteurized beer, which had previously been heated at 60°C for 30 min., was examined by the methylene blue test under the same condition as the preceding experiment. The solution was unchanged in color even after 3 days.

This proves the perfect loss of the dehydrase function in Pasteurized beer.

On the other hand, if the reaction-system (*) in the experiment (2) had previous been heated at 60°C for 30 min., methylene blue was not reduced and sunflavor was not given off by exposing the solution to sunlight.

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