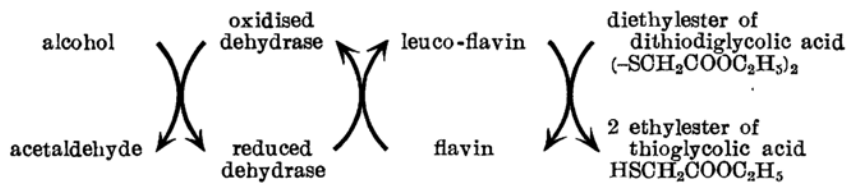
The Sunflavor of Beer. III. Mechanism of Sunflavor Development

By Tei YAMANISHI and Yataro OBATA

(Received February 2, 1950)

In the previous paper the authors reported that the development of the sunflavor in beer, exposed to the sunlight, was due to the reduction of diethylester of dithiodiglycolic acid (oxidised-type) to ethylester of thioglycolic acid (reduced-type). This is produced by the following reaction system.



By further investigation in the direction of the absorption-spectra, it was found that the flavin was activated by the sunlight, particularly by the rays ranging from 441 to 452 mμ, and then the reaction of this system was accelerated.

A few works has appeared in the literature relating to the light-effect on dehydrase system, and it was reported by all workers that the light activates the dehydrase system.[1][2][3]

According to the results of the authors' experiments on the absorption-spectra of the visible rays with the flavin and the dehydrase, the maximum absorption of the flavin takes place at the rays ranging from 441 to 452 mμ. in the wave length, as far as the rays which can pass the glass plate are concerned, while the dehydrase shows no special absorption band.

On the other hand, there are two kinds of beer bottles; one is made of brown colored glass and the other green colored glass. That the sunflavor developed more slowly in the brown bottle than in the green bottle was actually observed in a model experiment as well as in the bottled raw-beer on the market. By studying the absorption-spectra of these two kinds of bottles, it was found that the brown bottle absorbed the rays with the wave length smaller than 452.5 mμ, while the green bottle absorbed the rays with the wave length ranging from 523 to 561 mμ, as well as those with the wave length smaller than 406 mμ. Then it is concluded that the rays which are concerned with the development of sunflavor have the wave length ranging from 406 to 452 mμ. This range of the wave length coincides in general with the range in which the maximum absorption of the flavin takes place. To prove the effect of the rays in this range of the wave length the authors have carried out a model experiment by exposing the beer to the light filtered through a yellow glass plate which does not pass the rays in question and a violet glass plate which passes them exclusively. The result has proved just as the authors had expected that the

(1) Hans von Euler, *Z. phisiol. Chem.*, **232**, 16 (1935).
(2) H. v. Euler and E. Klussmann, *Naturwiss.* **22**, 777 (1934).
(3) Krestownikoff, *Skand. Arch. für Phys.* **52**, 199 (1927).

development of the sunflavor of beer was more rapid when the violet filter was employed.

From these results it has become clear that the sunlight activates the flavin and in consequence of this the sunflavor of beer develops.

Experimental Part

The components of the reaction system, used in the model experiment to prove the mechanism of the sunflavor development in beer, are as follows:

Dehydrase solution*	5 cc.
Flavin**	0.3 mg.
Diethylester of dithiodiglycolic acid	0.1 g.
Ethyl alcohol	0.3 cc.
Phosphate buffer (pH 7.2)	5 cc.

* Prepared according to the method described in the second part of this report.

** Preparation of B_2 for injection, manufactured by the Sankyo Co.

Model Experiment with the Green and Brown Bottles.—A test tube filled with the solutions of the above mentioned reaction system and tightly closed with a rubber-stopper is hanged with a string in each of the bottles with close stoppers. These bottles are exposed to the direct summer sunlight. The air temperature in the closed bottles rises to 44-45°. The result of the experiment is shown in Table 1.

Table 1

The Result of Model Experiment with Green and Brown Bottles

Color of bottle	Exposure 3 hrs.	5 hrs.	9 hrs.
brown	almost no change (−)	flavin is slightly discolored (−)	flavin is considerably discolored (±)
green	almost no change (−)	flavin becomes colorless (±)	flavin becomes considerably colorless (+)

Note: (+) and (−) denote respectively the high and low extent of the sunflavor development.

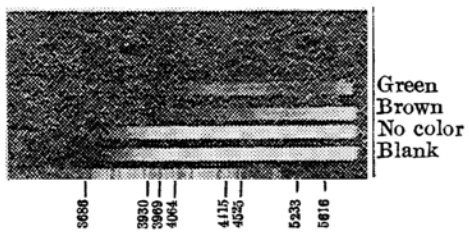


Fig. 1.—The absorption-spectra of the beer-bottles.

As we can learn from Fig 1. the green bottle absorbs the rays with the wave length ranging from 523-561 mμ as well as those with the wave length smaller than 406 mμ, and the brown bottle absorbs the rays with the wave length smaller than 452 mμ.

The Absorption-Spectra of the Flavin and the Dehydrase.—The absorption-spectra of the flavin and the dehydrase solutions are shown in Figs. 2 and 3 respectively.

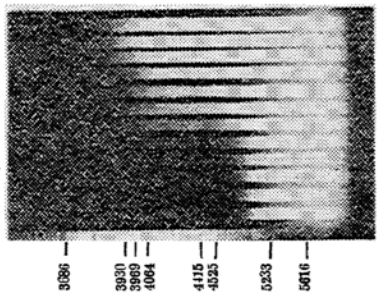


Fig. 2.—The absorption-spectra of the flavin.

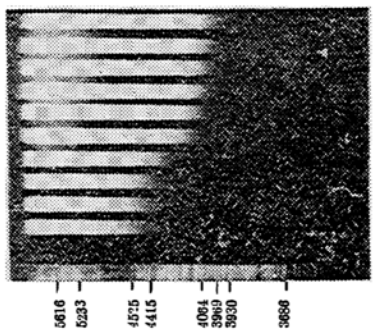


Fig. 3.—The absorption-spectra of the dehydrase.

As it is clearly shown in these figures, the absorption bands of the flavin are situated at the position of the rays with the wave length ranging from 441 to 452 mμ (Maximum absorp-

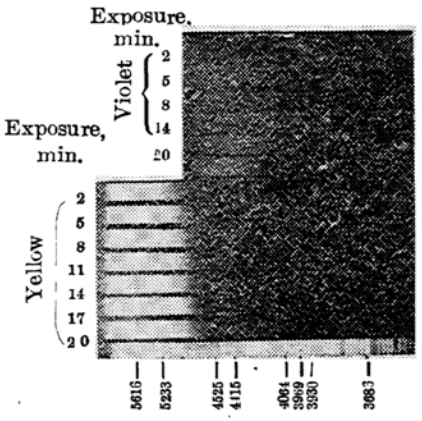


Fig. 4.—The absorption-spectra of the filters

tion band at 440 mμ) and of the rays with the wave length smaller than 388 mμ. The dehydrase has no special absorption band.

The Effect of the Sunlight Filtered through the Yellow and Violet Glass Plates.—The absorption spectra of the colored glass plates used as the filter are shown in Fig. 4.

The violet glass filter passes exclusively the rays with the wave length ranging from 453 mμ to 406 mμ or the rays in which the maximum absorption bands of the flavin are situated. The yellow glass filter absorbs entirely the rays with the wave length smaller than 453 mμ.

The reaction system was exposed for five hours to the sunlight filtered through each of these colored glass plates, and then the development of sunflavor was examined. Under the violet filter the color of the solution faded away into slight as the flavin changed to leucoflavin and a slight development of sunflavor was recognised. Under the yellow filter, on the other hand, the solution maintained the yellow color of flavin and the sunflavor could not be detected.

The result of the above experiment is shown in Table 2.

From the results of these experiments it has become clear that the rays which have concern with the development of sunflavor in beer are those in which the flavin has its maximum absorption and range from 453 to 406 mμ in the wave length.

The development of sunflavor in beer exposed to the sunlight is thus due to the activation of the flavin by the light.

Table 2
The Effect of the Filtered Sunlight

filter color	Exposure		
	3 hrs.	5 hrs.	9 hrs.
violet	almost no change	flavin becomes leucoflavin (±)	flavin becomes leucoflavin (+)
yellow	„	almost no change (−)	flavin is slightly discolored (±)

In these experiments it took a very long time to get the development of sunflavor. This is considered to be owing to the free flavin employed. If the flavin enzyme is used instead, the results will probably be obtained more rapidly and distinctly.

The authors are now under research to solve the problem concerned.

The expenses of the present study was partly defrayed from the Scientific Research Encouragement Fund granted by the Ministry of Education for which the authors thanks are due.

Institute of Agricultural Chemistry,
Hokkaido University, Sapporo