Influence of Ultraviolet Irradiation on Spores of Obligate Anaerobes Causing Flat Sour Spoilage of Canned Drinks*

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INTRODUCTION

Previous papers[1-4] have described a new type of flat sour spoilage (O.A. flat sour spoilage) in commercial canned coffee and *shiruko* which were kept hot in vending machines, and it was reported that the heat resistance of the causative bacteria is so high that the conventional heating process is insufficient to sterilize the canned drinks. Sugar, which is the only common ingredient used in both canned coffee and *shiruko*, is most strongly suspected as a source of the causative bacteria[4].

Therefore, one possible approach to prevent O.A. flat sour spoilage is previous sterilization of the sugar by some means other than thermal processes. For this purpose, ultraviolet (UV) irradiation seems to be the only convenient and effective method. In this paper, the UV resistance and the effects of UV irradiation on the heat resistance of the spores are reported.

Table 1. Characteristics of the Strains

Strain No.	24-1	13-1	26-11	27-8	28-3	28-4
Origin	Canned coffee		Canned coffee		Canned *shiruko*	
(manufacturer)	(B)	(F)	(J)	(J)	(K)	(K)
Heat resistance (D_{120})	25	5.6	23	25	46	22
Gram stain	−	−	−	−	−	−
Acid from						
glucose	+	+	+	+	+	+
lactose	−	−	−	−	−	−
salicin	−	−	−	−	−	−
sucrose	−	−	−	−	−	−
Production of						
indole	−	−	−	−	−	−
H_2S	+	+	+	+	+	+
Coagulation of						
milk	−	−	−	−	−	−
Digestion of						
albumin	−	−	−	−	−	−
meat	−	−	−	−	−	−
Nitrite from						
nitrate	−	−	+	−	+	−
Hydrolysis of						
gelatin	−	+	−	−	−	−

* A New Type of Flat Sour Spoilage — IV.

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MATERIALS AND METHODS

Microorganisms

The bacteria tested were the six isolates (strain Nos. 24-1, 13-1, 26-11, 27-8, 28-3 and 28-4) obtained as the causative bacteria of O.A. flat sour spoilge. Strain Nos. 24-1 and 13-1 were isolated from spoiled canned coffee samples from manufacturers B and F, respectively[2]. Strain Nos. 26-11 and 27-8 were isolated from spoiled canned coffee samples from manufacturer J[3]. Strain Nos. 28-3 and 28-4 were isolated from spoiled canned *shiruko* samples from manufacturer K[4]. The principal characteristics of the strains are shown in Table 1.

Bacillus subtilis 3610 (ATCC 6051) was used as a standard in comparing the UV resistance and heat resistance of these strains. The strain was obtained from Prof. Hajime Kadota, Faculty of Agriculture, Kyoto University.

Preparation of spore suspensions

Spore suspensions were prepared by the method described in the previous paper[2] except for *Bacillus subtilis.*

Bacillus subtilis was grown at 35°C on the standard method agar (SMA)[5]. Spores were harvested after 4 days of incubation and suspended in sterile deionized water of the same volume as the medium. The suspension was washed and treated by the method described previously[2] without heat treatment.

UV irradiation

The UV-light source used was a germicidal lamp (Hitachi GL-15, 15W) emitting predominantly 2537Å radiation. Dose rates were measured with a Topcon UVR-254 UV radiometer (TOKYO KOGAKU KIKAI Co., Ltd.).

Aliquots (2ml each) of the spore suspensions were irradiated in a 9.5-cm Petri dish at a dose rate of 400 or 500μW/cm^2. During UV irradiation, the Petri dish was rotated slowly to ensure uniform irradiation.

Heating

The spores of the strains causing O.A. flat sour spoilage irradiated as described above were heated at 120±0.1°C by the method described previously[2].

The spores of *Bacillus subtilis* were heated at 90, 95 and 100°C by the method described previously[2].

Determination of survivors

The six strains causing O.A. flat sour spoilage were irradiated and heated for desired periods, then the numbers of survivors were determined by the MPN method with mTGC[6]; in the case of *Bacillus subtilis* the numbers of survivors were determined by the pour-plate method with SMA. The mTGC was incubated at 55°C for 10 days and the SMA was incubated at 35°C for 10 days.

RESULTS AND DISCUSSION

UV resistance

UV survival curves of the 6 strains causing O.A. flat sour spoilage are shown in Fig. 1. From

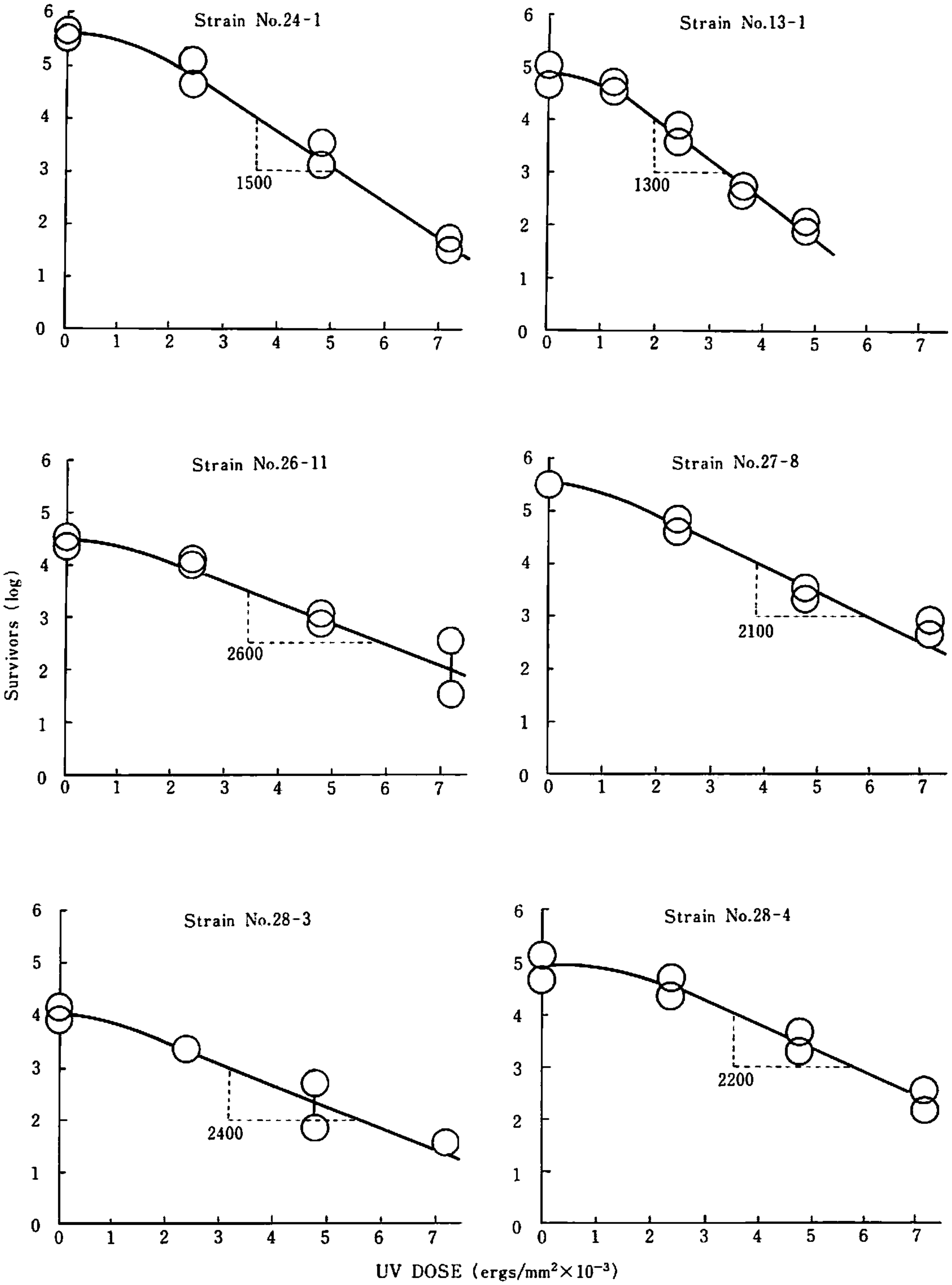

Fig. 1. UV Survival Curves for Spores of the Six Strains Causing O.A. Flat Sour Spoilage
Numerals are D_{UV} values.

these UV survival curves, the D_{uv} values, which are defined as the UV doses required to reduce the numbers of survivors to one-tenth of the original populations, were determined. The D_{uv} values ranged from 1300 to 2600 ergs/mm². These values are not especially high as compared to the known values for other spores[7] and the value for the spores of *Bacillus subtilis* (1100 ergs/mm²) obtained by the same method (Fig. 2). However, the heat resistance of the spores of *Bacillus subtilis* was very low. The D values at 100, 95 and 90°C (D_{100}, D_{95} and D_{90}) were 0.4, 0.9 and 6.5, respectively, as shown in Fig. 3. It is considered that, estimating from the z-value (8.3°C), these values were 1/8600–1/71000 of those of the spores of the six strains.

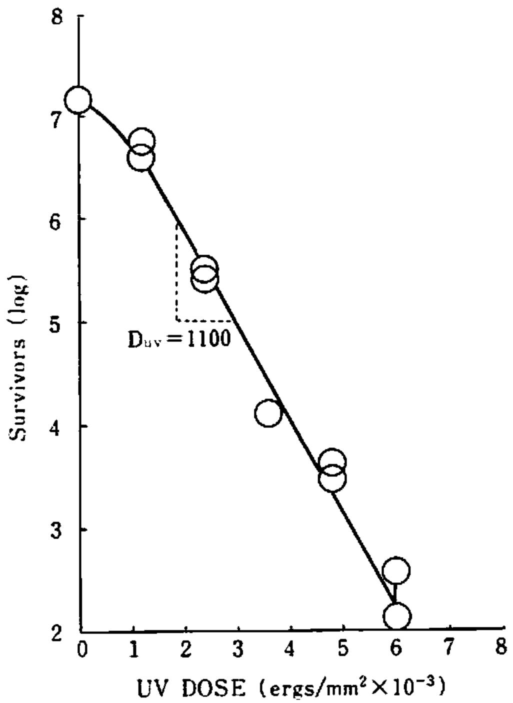

Fig. 2. UV Survival Curve for Spores of *Bacillus subtilis*

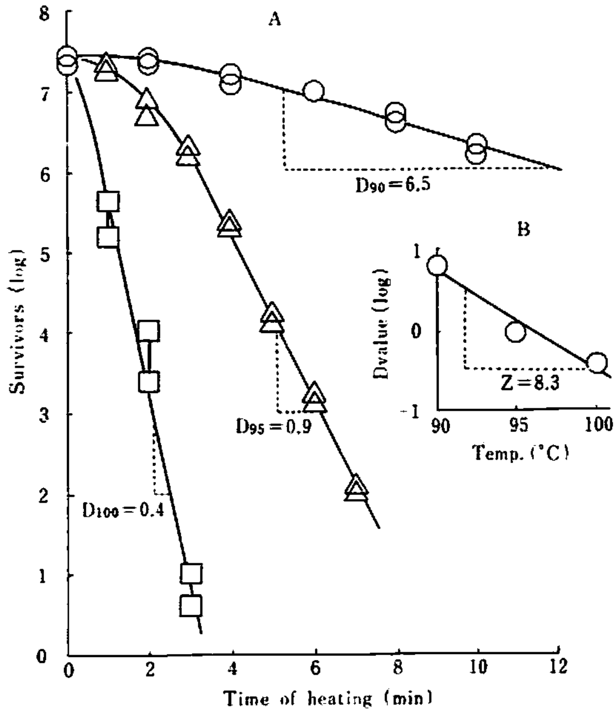

Fig. 3. Heat-survival Curves and Thermal Destruction Curve for Spores of *Bacillus subtilis*
A. Heat-survival curves:
—○— , heated at 90°C;
—△— , heated at 95°C;
—□— , heated at 100°C.
B. Thermal destruction curve.

Table 2. Heat Resistance and UV Resistance

Strain No.	Heat resistance D_{120} value	UV resistance Duv value*1
24-1	25	1500
13-1	5.6	1300
26-11	23	2600
27-8	25	2100
28-3	46	2400
28-4	22	2200
Bacillus subtilis	0.4*2	1100

*1 UV dose required to reduce the number of the survivors to one-tenth of the original population.
*2 D value at 100°C.

The D values at 120°C (D_{120}) reported previously[2–4] and the D_{uv} values for the spores of the strains are summarized in Table 2. The D_{120} value for the spores of strain No. 28-3 was 8.2 times larger than that for the spores of strain No. 13-1. However, strain No. 28-3 showed a D_{uv} value 1.8 times that of strain No. 13-1. Moreover, the D_{120} value for the spores of strain No. 28-3 was about 40000 times that for the spores of *Bacillus subtilis* as estimated from the z-value. However, strain No. 28-3 showed a D_{uv} value only 2.2 times greater than that of *Bacillus subtilis.*

These results indicate that there is no relation between the UV resistance and the heat resistance of these spores, and that, unlike the heat resistance, the UV resistance of these 6 strains is not especially high.

Effects of UV irradiation on heat resistance

Heat survival curves for the spores irradiated at the dose rate of 500μW/cm^2 for 30, 60 and 90 sec are shown in Figs. 4, 5, 6, 7, 8 and 9. In the figures, each symbol is the mean value of the data obtained in duplicate experiments. The differences between the D_{120} values for the spores of some strains and those for the spores of the same strains given in the previous paper[2–4] and in Tables 1 and 2 are due to the fact that spores of different batches were used.

The D_{120} value for the spores of strain No. 24-1 was 25, and the D_{120} values for the spores irradiated for 30, 60 and 90 sec were 17, 19 and 17, respectively (Fig. 4). The results suggest that, in the range of UV dose used, the D_{120} values were decreased to 70% of the original levels by the UV irradiation. However, no significant changes were observed with change of the UV dose.

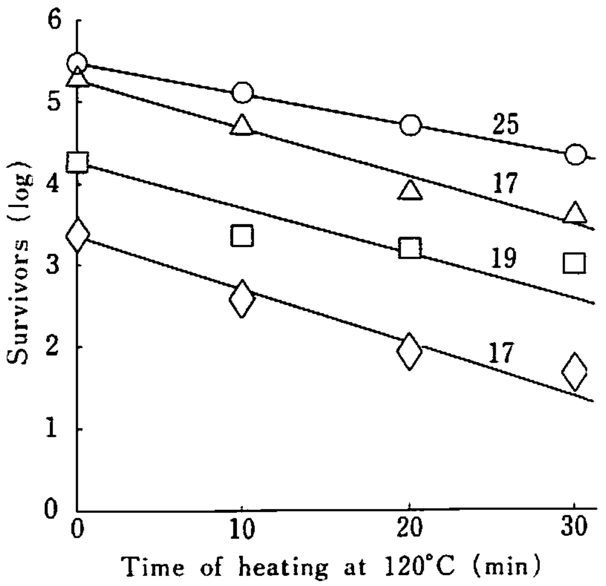

Fig. 4. Effects of UV Irradiation on the Heat Resistance of Strain No. 24-1
Spore suspensions were irradiated at 500μw/cm^2 for 0 sec (control, —○—), for 30 sec (—△—), for 60 sec (—□—), and for 90 sec (—◇—), and subjected to heat treatment at 120°C.
Numerals on the plots are D_{120} values.

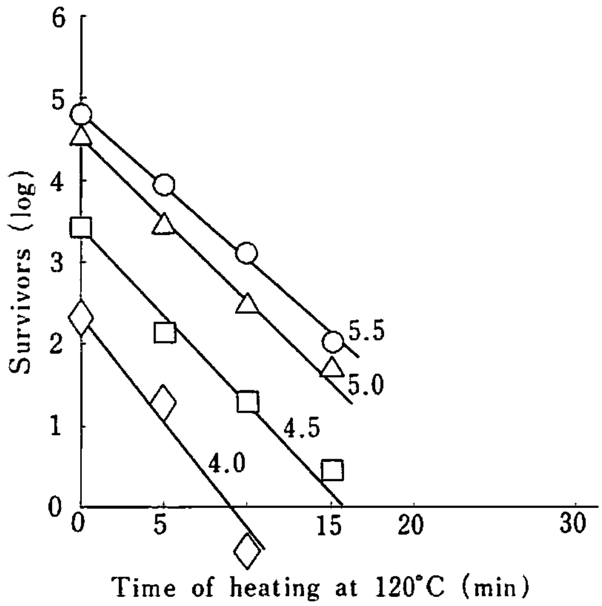

Fig. 5. Effects of UV Irradiation on the Heat Resistance of Strain No. 13-1
Spore suspensions were irradiated at 500μw/cm^2 for 0 sec (control, —○—), for 30 sec (—△—), for 60 sec (—□—), and for 90 sec (—◇—), and subjected to heat treatments at 120°C.
Numerals on the plots are D_{120} values.

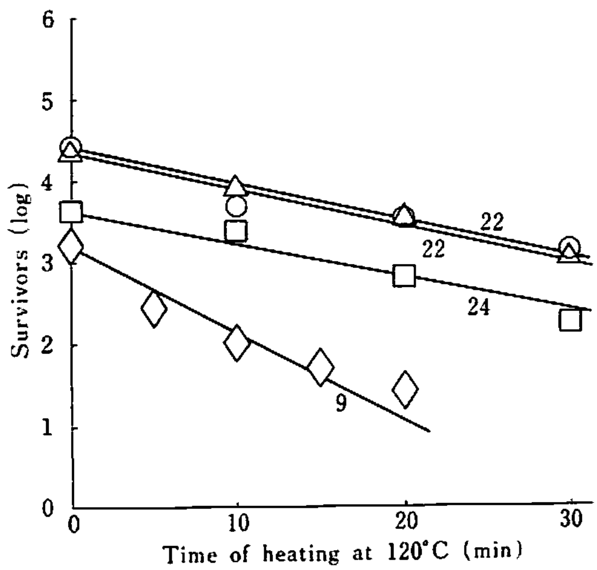

Fig. 6. Effects of UV Irradiation on the Heat Resistance of Strain No. 26-11
Spore suspensions were irradiated at 500 μw/cm² for 0 sec (control, —○—), for 30 sec (—△—), for 60 sec (—□—), and for 90 sec (—◇—), and subjected to heat treatment at 120°C.
Numerals on the plots are D_{120} values.

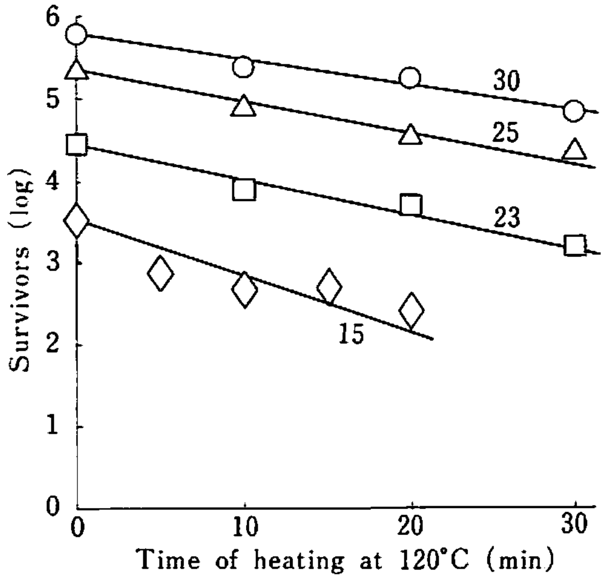

Fig. 7. Effect of UV Irradiation on the Heat Resistance of Strain No. 27-8
Spore suspensions were irradiated at 500 μw/cm² for 0 sec (control, —○—), for 30 sec (—△—), for 60 sec (—□—), and for 90 sec (—◇—), and subjected to heat treatments at 120°C.
Numerals on the plots are D_{120} values.

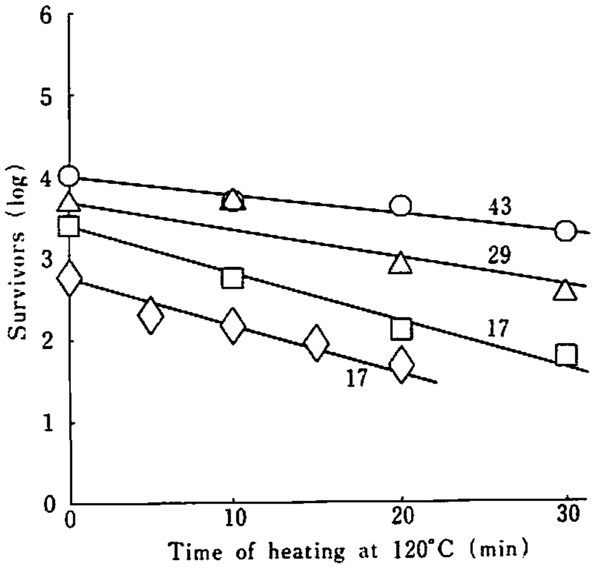

Fig. 8 Effects of UV Irradiation on the Heat Resistance of Strain No. 28-3
Spore suspensions were irradiated at 500 μw/cm² for 0 sec (control, —○—), for 30 sec (—△—), for 60 sec (—□—), and for 90 sec (—◇—), and subjected to heat treatment at 120°C.
Numerals on the plots are D_{120} values.

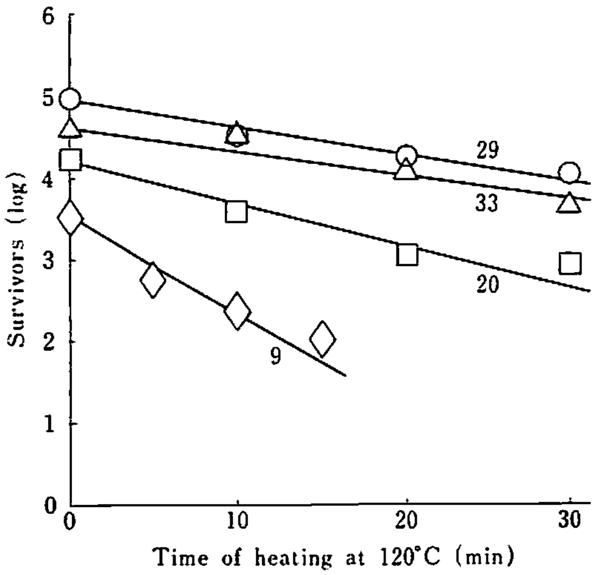

Fig. 9. Effects of UV Irradiation on the Heat Resistance of Strain No.28-4
Spore suspensions were irradiated at 500 μw/cm² for 0 sec (control, —○—), for 30 sec (—△—), for 60 sec (—□—), and for 90 sec (—◇—), and subjected to heat treatment at 120°C.
Numerals on the plots are D_{120} values.

The D_{120} value for the spores of strain No. 13-1 was 5.5, and decreased to 5.0, 4.5 and 4.0 with increase of the UV dose (Fig. 5).

The D_{120} value for the spores of strain No. 26-11 was 22, and after UV irradiation at 500μW/cm^2 for 30 sec no significant decrease in the number of the spores or in the D_{120} value was observed. Even after 60 sec of UV irradiation, the D_{120} value was not changed, but after 90 sec it decreased to one-half (Fig. 6).

The D_{120} value for the spores of strain No. 27-8 was 30, and decreased to 25, 23 and 15 with increase of the UV dose (Fig. 7).

The D_{120} value for the spores of strain No. 28-3 was 43, and decreased to 29, 17 and 17 with increase of the UV dose (Fig. 8).

The D_{120} value for the spores of strain No. 28-4 was unchanged after UV irradiation for 30 sec, and after UV irradiation for 60 and 90 sec, the values decreased to two-thirds and one-third, respectively (Fig. 9).

Judging from these results, in the range of UV doses used, the six strains can be divided into three types as regards the effects of UV irrradiation on the heat resistance.

In the first type, strain No. 24-1, UV irradiation decreases the heat resistance, but an increase of the UV dose does not cause any further significant change in the heat resistance.

In the second type, strain Nos. 13-1, 27-8 and 28-3, the heat resistance decreases with increase of the UV dose.

In the third type, strain Nos. 26-11 and 28-4, the heat resistance is not changed by a limited UV dose, but is greatly decreased by a larger dose.

From these results on the UV resistance and the effects of UV irradiation on the heat resistance, it is concluded that the spores of the six strains causing O.A. flat sour spoilage do not have an especially high resistance to UV light, in contrast to their heat resistance, and at least a half of the original heat resistance is lost upon UV irradiation.

It is suggested that prior UV irradiation of the dissolved sugar can be employed as a countermeasure to prevent O.A. flat sour spoilage of canned drinks which are often kept hot in vending mechines in Japan.

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