

## A New Type of Flat Sour Spoilage of Commercial Canned *Shiruko* \*

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### INTRODUCTION

A new type of flat sour spoilage (O.A. flat sour spoilage) of commercial canned coffee, which had been kept hot in vending machines, was described in previous reports<sup>1-3</sup>). It is said that a similar phenomenon also takes place in commercial canned *shiruko* (a sweet bean drink) which is also kept and drunk hot.

This paper deals with an investigation of canned *shiruko* taken from a lot which had suffered from flat sour spoilage.

### MATERIALS AND METHODS

#### Samples of canned *shiruko*

Sixty-four cans were taken from a lot of commercial canned *shiruko* which had suffered from flat sour spoilage. It had been packed on October 18, 1978, by the manufacturer K. The net weight of the cans was 195 g.

#### Incubation test

Sixty cans were divided into two groups, each containing 30 cans, and incubated at 55°C. The first group was tested after 10 days and the second group after 30 days.

After each period, the pH values and vacuum levels of 15 cans were measured, and the other 15 cans were opened under aseptic conditions and tested microbiologically. The pH values of the contents of the latter were also measured.

As controls, the remaining four cans were incubated as room temperature. Two cans were subjected to vacuum level and pH measurements after 10 days and after 30 days.

#### Isolation of bacteria from the cans

Bacteria were detected and isolated from the cans, and purified by the methods described previously<sup>2,3</sup>).

Biochemical examination, preparation of spore suspensions of the isolated bacteria, and determinations of D values of the spores at 120°C, and of sulfite- and sulfate-reducing abilities

These experiments were carried out by the methods described in the previous papers<sup>2,3</sup>).

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\* A New Type of Flat Sour Spoilage — III.

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## RESULTS AND DISCUSSION

## Incubation test

No swollen cans were found under any of the conditions of incubation.

The pH values and vacuum levels of the cans are shown in Table 1. After storage for 10 days at 55°C, six spoiled cans were found and the spoilage rate was 20%. The pH values and vacuum levels ranged from 4.9 to 5.6 and from 28 to 31 cmHg, respectively, while those of normal cans ranged from 5.6 to 6.1 and from 25 to 35 cmHg, respectively. After 30 days, all the cans were spoiled and the spoilage rate was 100%. The pH values and vacuum levels ranged from 4.7 to 5.5 and from 16 to 34 cmHg, respectively.

After storage at room temperature for 10 and 30 days, the pH values of the controls were 6.2 and 6.1, respectively. The vacuum levels of the controls after 10 and 30 days were about 31 and 32 cmHg, respectively.

Table 1. PH values and vacuum levels of canned *shiriko*

Incubation		Can No.	Vacuum	pH	Incubation		Can No.	Vacuum	pH
Days	Temp.				Days	Temp.			
10	55°C	28-1- 1*	28	5.6	30	55°C	28-3- 1*	32	5.0
		28-1- 2*	28	5.3			28-3- 2*	33	5.0
		28-1- 3	32	6.0			28-3- 3*	30	4.9
		28-1- 4*	31	4.9			28-3- 4*	16	5.0
		28-1- 5	35	6.0			28-3- 5*	34	4.8
		28-1- 6	33	6.1			28-3- 6*	28	4.9
		28-1- 7	34	6.0			28-3- 7*	30	4.8
		28-1- 8	26	6.1			28-3- 8*	29	4.9
		28-1- 9	34	6.1			28-3- 9*	27	4.9
		28-1-10	33	6.0			28-3-10*	34	4.9
		28-1-11	29	6.0			28-3-11*	31	4.9
		28-1-12	35	5.9			28-3-12*	29	5.0
		28-1-13	25	6.0			28-3-13*	33	4.9
		28-1-14	34	5.9			28-3-14*	28	5.0
		28-1-15	32	6.0			28-3-15*	30	4.8
		28-1-16		6.0			28-3-16*		4.8
		28-1-17		6.1			28-3-17*		4.7
		28-1-18		6.0			28-3-18*		4.9
		28-1-19		6.0			28-3-19*		4.8
		28-1-20		6.0			28-3-20*		5.0
		28-1-21		6.1			28-3-21*		4.8
		28-1-22*		5.3			28-3-22*		5.0
		28-1-23*		5.4			28-3-23*		4.9
		28-1-24		6.0			28-3-24*		4.9
		28-1-25		6.0			28-3-25*		4.8
		28-1-26		6.0			28-3-26*		4.9
		28-1-27		6.0			28-3-27*		4.9
		28-1-28		6.0			28-3-28*		5.5
		28-1-29*		5.3			28-3-29*		4.7
		28-1-30		6.0			28-3-30*		5.0
Room temp.		28-1-31	30	6.2	Room temp.		28-3-31	34	6.1
		28-1-32	32	6.2			28-3-32	32	6.1

\* Spoiled cans.

The contents of the spoiled cans were reddish or yellowish (the normal color is russet), and had a slight smell of hydrogen sulfide and a slight bitterness.

The decrease of the pH values of the spoiled canned *shiruko* was about 1.0. However, in contrast to the flat sour spoilage of canned coffee<sup>2,3)</sup>, no significant change of the vacuum level was observed.

The spoilage ratio increased to 100% after 30 days from 20% after 10 days. Therefore, all the cans had contained spores of the causative bacteria, and the spoilage proceeded throughout the storage period. Thus, all the cans opened under aseptic conditions after 10 days were tested microbiologically, whether they were spoiled or not.

#### Isolation of bacteria from the cans

Table 2 shows the results of tests for the detection of bacteria from the cans opened under aseptic conditions with eight kinds of media at 35°C and at 55°C. No bacteria were detected from any of the cans with mTGC or SMA incubated at 35°C, or with DTA or SMA incubated at 55°C.

Table 2. Detection of bacteria from the cans<sup>\*1</sup>

Incubation temp. Can No. \ Media	35°C		55°C							
	mTGC <sup>*2</sup>	SMA <sup>*3</sup>	mTGC <sup>*2</sup>	ISA <sup>*4</sup>	ISaA <sup>*4</sup>	TF <sup>*4</sup>	TSiF <sup>*4</sup>	TSaF <sup>*4</sup>	DTA <sup>*3</sup>	SMA <sup>*3</sup>
28-1-16	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-1-17	—,—	0,0	+,+	+,+	—,—	—,—	—,—	—,—	0,0	0,0
28-1-18	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-1-19	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-1-20	—,—	0,0	+,+	+,+	—,—	—,—	—,—	—,—	0,0	0,0
28-1-21	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-1-22	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-1-23	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-1-24	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-1-25	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-1-26	—,—	0,0	+,+	+,+	—,—	—,—	—,—	—,—	0,0	0,0
28-1-27	—,—	0,0	+,+	+,+	—,—	—,—	—,—	—,—	0,0	0,0
28-1-28	—,—	0,0	+,+	+,+	—,—	—,—	—,—	—,—	0,0	0,0
28-1-29	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-1-30	—,—	0,0	+,+	+,+	—,—	—,—	—,—	—,—	0,0	0,0
28-3-16	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-3-17	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-3-18	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-3-19	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-3-20	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-3-21	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-3-22	—,—	0,0	+,+	—,—	—,—	—,—	—,—	—,—	0,0	0,0
28-3-23	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-3-24	—,—	0,0	+,+	—,—	—,—	—,—	—,—	—,—	0,0	0,0
28-3-25	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-3-26	—,—	0,0	+,+	—,—	—,—	—,—	—,—	—,—	0,0	0,0
28-3-27	—,—	0,0	+,+	—,—	—,—	—,—	—,—	—,—	0,0	0,0
28-3-28	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-3-29	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-3-30	—,—	0,0	+,+	—,—	—,—	—,—	—,—	—,—	0,0	0,0

\*1 Duplicate examinations.

\*2 + : growth of bacteria was indicated by the turbidity of the media, — : no growth.

\*3 Numbers of colonies.

\*4 Blackening of the media was observed in some cases, + : blackened, — : not blackened.

Some bacteria were found to grow in mTGC incubated at 55°C.

In ISaA, TF and TSaF, blackening was not observed. In ISA and TSiF, blackening was observed, except in the case of ISA after 30-day incubation. It seems that long incubation repressed the growth of the bacteria in ISA.

These results clearly indicate that the bacteria causing the spoilage are thermophilic, anaerobic and sulfite-reducing, but not sulfate-reducing.

Some spore-forming obligate anaerobes were isolated from 16 out of 30 cans. These 16 cans are shown in Table 3. The can numbers are simplified in this table. A strain was selected randomly from isolates of each can and given the same number as the can from which it had been isolated. Sixteen strains were obtained.

Table 4 shows the results of the incubation of all the strains under the conditions described in Table 2. All the strains isolated were found to be the causative bacteria of the spoilage. It can be concluded that TSiF is better than ISA as a growth medium for the causative bacteria. These results are the same as those found in the previous papers<sup>2,3)</sup>.

Table 3. Cans from which strains were isolated

Can No.	Simplified No.	Can No.	Simplified No.
28-1-16	28- 1	28-1-28	28- 9
28-1-18	28- 2	28-1-29	28-10
28-1-21	28- 3	28-3-17	28-11
28-1-22	28- 4	28-3-19	28-12
28-1-23	28- 5	28-3-21	28-13
28-1-24	28- 6	28-3-25	28-14
28-1-25	28- 7	28-3-29	28-15
28-1-27	28- 8	28-3-30	28-16

Table 4. Incubation of isolates under the conditions used for the detection of bacteria from the spoiled cans<sup>\*1</sup>

Incubation temp. Strain No. \ Media	35°C		55°C							
	mTGC	SMA	mTGC	ISA	ISaA	TF	TSiF	TSaF	DTA	SMA
	*2									
28- 1	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28- 2	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28- 3	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28- 4	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28- 5	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28- 6	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28- 7	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28- 8	—,—	0,0	+,+	—,+	—,—	—,—	+,+	—,—	0,0	0,0
28- 9	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-10	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-11	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-12	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-13	—,—	0,0	+,+	+,+	—,—	—,—	+,+	—,—	0,0	0,0
28-14	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-15	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0
28-16	—,—	0,0	+,+	—,—	—,—	—,—	+,+	—,—	0,0	0,0

\*1 See Table 2.

\*2 Symbols are the same as in Table 2.

### Characteristics of isolates

Table 5 shows the characteristics of the strains isolated. All were Gram-negative and produced acids from glucose but not from lactose, sucrose or salicin. They produced hydrogen sulfide, but did not produce indole. The tests for coagulation of milk, digestion of albumin and meat, and hydrolysis of gelatin gave negative results.

Table 5. Characteristics of isolated strains

Strain No.	28- 2	28-15	28- 1	28-10
	28- 3	28-16	28- 4	28-11
	28- 6		28- 5	28-12
	28- 8		28- 7	28-13
	28-14		28- 9	
Gram stain		—		—
Acid from				
glucose		+		+
lactose		—		—
salicin		—		—
sucrose		—		—
Production of				
indole		—		—
H <sub>2</sub> S		+		+
Coagulation of				
milk		—		—
Digestion of				
albumin		—		—
meat		—		—
Nitrite from				
nitrate		+		—
Hydrolysis of				
gelatin		—		—
D <sub>120</sub> value	28- 3 : 46, 28- 8 : 13		28- 4 : 22, 28- 9 : 10	

However, the strains could be divided into two groups in terms of the ability to reduce nitrate. One group contained seven strains which reduced nitrate. The other contained nine strains which do not reduce nitrate.

The D values at 120°C of representative strains of each group were 13 and 46, and 10 and 22, respectively.

These characteristics of the strains are the same as those of the strains obtained from the spoiled canned coffee<sup>2,3</sup>.

These results indicate that the flat sour spoilage of canned *shiruko* is the same as the new type of flat sour spoilage (O.A. flat sour spoilage) of canned coffee described in the previous paper<sup>1-3</sup>.

On the assumption that the causative bacteria are introduced from one of the ingredients, sugar is the natural suspect, because it is the only common ingredient used in both canned coffee and canned *shiruko*.

This conclusion is consistent with the information provided by the Japanese packers, that the practical rates of flat sour spoilage of the canned drinks changed with the different lots of sugar used for manufacture.

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## References

- 1) Nakayama, A., Samo, S., Ikegami, Y.: Bull. Japan Soc. Sci. Fish., 43, 899 (1977).
- 2) Nakayama, A., Samo, S.: Bull. Japan. Soc. Sci. Fish., 46, 1117~1123 (1980).
- 3) Nakayama, A., Shinya, R.: J. Food Hyg. Soc. Japan., 22, 30~36 (1981).