CHAPTER 20

CONTAMINATION OF MUSHROOMS AND CANNED MUSHROOMS

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1. INTRODUCTION

Fresh and canned mushrooms are widely preferred by most people as nutritive foods. China exports more than 100,000 tons of edible mushrooms each year so it is very important to have quality control against various forms of contamination in order to protect the people's health and to benefit the consumers.

2. POSSIBLE SOURCES OF CONTAMINATION AND PREVENTIVE MEASURES

Mushrooms are very tender and easily damaged. For instance, if the picked fresh mushrooms are not refrigerated and processed in the proper time, air-borne microorganisms will grow on them, and endogenous oxidation will take place, causing a change in colour. In this way, the quality of mushrooms will deteriorate and the mushrooms are spoiled and become unacceptable for eating or processing. Contamination of canned mushrooms comes from two main sources. One is filth contamination. In this case, unacceptable contaminated mushrooms have been used or the mushrooms are not thoroughly washed, or sanitary measures are not practised during processing, so the contamination ends up in the finished products. Sometimes, this contamination may be very dangerous for the consumer. Another form is microbial contamination that arise for various reasons, e.g. the fresh mushrooms are transported within unclean containers; raw materials are laid up for long peroids and not processed in time; practices which did not meet the sanitary requirements for the environment and processing; can defects which are caused by machinery or poor practices; retort equipment and temperature recorders are not operated properly and/or the cooling water did not contain enough chlorine, and so on. In this way, air-borne microorganisms are introduced and allowed to flourish, deteriorating the mushroom products.

In order to guarantee the quality of mushrooms and canned mushrooms, the related departments and canneries have undertaken many measures to strengthen quality control. Specific regulations and rules have been made for the mushroom growing, collection, transportation, processing and storage. Training classes have been held to introduce some advanced equipment and to improve the testing methods. The "Good Manufacture Practice" (GMP) and "Hazard Analysis Critical Control Point" (HACCP), which are the scientific systems for maintaining quality control for food safety, have been set up. Simultaneously, the quality of Chinese canned mushrooms has improved.

3. NON-SPECIFIC REACTION OF THE TECRA KIT

When speaking about the contamination of canned mushrooms, people often think about the contamination by staphylococcal enterotoxins (SET). In 1989, the US Food and Drug Adminstration (FDA) declared SET contamination found in Chinese canned mushrooms, and they issued an order to automatically detain all canned mushrooms processed in China. At that time, the Chinese Government and the related departments responded promptly. The response was not only to take several efficient measures to keep the quality control for the canned mushrooms, but also to organize a scientific research group to study the reasons for SET contamination of canned mushrooms and to develop preventive measures.

At the beginning, we adopted the TECRA Kit which is used and recommended by the FDA to detect SET. We expected that SET would be found in the canned mushroom products. However, we often found that the results initially appeared positive but, after incubation for a while, turned negative. Sometimes, when using the TECRA Kit to examine mushrooms or material from the growing mushrooms, a very strong positive reaction could be changed to negative after treating the extracts with normal animal (goat, rabbit) serum. Another important observation was that one bacterium, *Serratia marcescens,* which does not produce SET, was isolated and gave a strong positive reaction with the TECRA Kit. So we doubt the specificity of the TECRA Kit. Through different experiments, especially comparisons of the specificity among four commercial screening kits, the nonspecific reaction of the TECRA Kit used for the detection of SET was confirmed (Wu *et al.*, 1992).

4. SPECIFICITY OF FOUR COMMERCIAL SCREENING KITS

The four test kits for detection of SET are as follows:

i. TECRA Kit (Bioenterprises Pty. Co., Australia), (Anon., 1990),
ii. SET-PRLA Kit (Oxoid Co., U.K.), (Shingaki *et al.*, 1990),
iii. TRANSIA Detection of SET Kit (TRANSIA Co., France), (Drouet, 1991),
iv. SET-EIA Kit (Brommelia Bern, Switzerland for Toxin Technology Inc., U.S.A.). (Bommell, 1990).

Two bacterial strains, *Serratia marcescens* and *Staphylococcus aureus*, were cultured in nutrient broth at 37°C for 48hr. The culture was then centrifuged and the supernatant divided into two portions: one was diluted with an equal volume of normal rabbit serum and stored at 37°C for 1 hr while the other portion was diluted with Tris buffer and stored at 37°C for 1 hr. Afterwards, the two portions were added to the four SET test kits, respectively. The results are showed in Table 1. All of the four SET test kits gave a positive result for the *S. aureus* culture and the results were not affected by animal serum. Only the TECRA Kit gave a positive result for *S. marcescens* and the

Table 1. Specificity of the Commercial Kits.

	TECRA-KIT		SET-RPLA		TRANSIA		SET-EIA	
Bacteria	Buffer 1:1	Serum 1:1	Buffer 1:1	Serum 1:1	Buffer 1:1	Serum 1:1	Buffer 1:1	Serum 1:1
S. aureus	+	+	+	+	+	+	+	+
S. marcescens	+	-	-	-	-	-	-	-

positive result turned negative after treatment with serum. This indicates that the cross-reaction of TECRA Kit with *S. marcescens* exists even though *S. marcescens* does not produce SET.

5. DETECTION OF SET IN MUSHROOM PRODUCTS USING TEST KITS

Cultivated material from three different mushroom growing areas were diluted with Tris buffer (1:4), homogenized and centrifuged. After centrifugation, the supernatant was filtered and divided into two portions. Subsequent work-up procedures were the same as described in Part 4. The results are shown in Table 2.

All three samples gave a positive reaction with the TECRA Kit. However, after treatment with serum, the samples gave negative results. Meanwhile, all three samples gave negative results with the other SET test kits. Based on these results, it is concluded that the substances in the cultivated materials which caused the positive reaction with the TECRA Kit were not SET. Cultivated mushroom material from Canada also gave a positive result with the TECRA Kit as did the two microbial species *Pseudomonas maltophilia* and a *Kluyveromyces* spp.isolated from the Canadian mushroom products. Therefore, it is suggested that substances which cause cross-reactions with the TECRA Kit also exist in cultivated mushroom material both from within and outside China.

Extracts from the same canned mushrooms which tested positive in the first detection by the TECRA Kit were re-examined. All the results were negative. However, if mushroom broth inoculated with staphylococcal enterotoxin A (2ng/ml) was stored under the same conditions and examined after a 4-week interval, it consistently gave a positive result. On examination of extracts from canned mushrooms using the TECRA Kit, we often found that the results were initially positive but turned negative when a second test was carried out within a 24 hr period. These results suggest that the positive TECRA reaction of true staphylococcal enterotoxin is stable but that of the cross-reacting

Table 2. Detection of SET in Mushrooms.

	TECRA-KIT		SET-RPLA		TRANSIA		SET-EIA	
Location	Buffer 1:1	Serum 1:1	Buffer 1:1	Serum 1:1	Buffer 1:1	Serum 1:1	Buffer 1:1	Serum 1:1
Shanghai	+	-	-	-	-	-	-	-
Fuzhou	+	-	-	-	-	-	-	-
Sichuan	+	-	-	-	-	-	-	-

substances is not.

The non-specific reaction of the TECRA Kit was confirmed not only in China, but also in other countries (Park *et al.*, 1992). So far, there are at least seven micro-organisms other than *S. aureus* that have been isolated from swollen cans or some other materials related to mushrooms or mushroom products, and which show positive results with the TECRA Kit. Therefore, the data described above cast doubt on the validity of decisions to ban the import of canned mushrooms from China based solely on results obtained using the TECRA Kit.

6. AN INVESTIGATION OF POTENTIAL SOURCES OF SET CONTAMINATION IN CHINA

In order to establish the possibility of SET contamination of canned mushrooms produced under the present Production Technology Procedure in China, samples were collected and examined from different processing steps such as: growing mushrooms, freshly picked mushrooms (before blanching), half-processed products (after blanching and before retortion), and finished products (after retortion).

S. aureus producing SET was intentionally inoculated into samples which showed a negative result with the TECRA Kit. Then, the population of *S. aureus* and the production of SET was followed.

6.1. Materials and Procedures

Materials: a) SET Testing Kit; TECRA Kit (Bioenterprise Pty Co., Australia)
b) Organon Micro ELISA system
c) *S. aureus* strain producing Enterotoxin A (provided by the Food Research Institute, University of Wisconsin)
Serratia marcescens, isolated by the Fujian Import and Export Commodity Inspection Bureau and identified by the China Identification Institute for Medical and Biochemical Products.
Escherichia coli and a *Proteus* sp., provided by the Fujian Health and Quarantine Station.
d) Cultivated mushroom samples: fresh, half-processed and canned.

Methods:
1. Deliberate contamination was carried out by adding SET-positive *S. aureus* to cultivated mushroom material which showed a negative SET result with the TECRA Kit. Also, a TECRA-positive SET solution was added to the roots of growing mushrooms. After the mushrooms had been grown under normal laboratory cultivation conditions, the fruit bodies were picked, washed and tested for SET using the TECRA Kit.
2. The growth and decline of *S. aureus*, and SET production, in cultivated mushroom material inoculated with 2.7×10^6 cells/g and maintained at 25°C was examined at 24h intervals.
3. The growth and decline of *S. aureus*, and SET production, was also examined in freshly picked mushrooms. Fresh mushrooms were inoculated with 1.75×10^7 cells/g, tied off in plastic bags, incubated at 25°C and the parameters measured at 6 h intervals.
4. Half-processed mushrooms, after blanching and before retortion, were inoculated with *S. aureus* (10^3 cells/g), incubated at 28°C and examined for SET at 4 h intervals.
5. Contamination of finished canned mushrooms: 10 cans were deliberately damaged by dropping from a height of 1 metre, 5 of the cans were soaked in a suspension of *S. aureus* and the remainder in tap water for a period of 4-6 h. The cans were then incubated at 37°C for 10 days and the contents examined for SET using the TECRA Kit.

Canned mushrooms were also contaminated with a mixture of microorganisms by drilling a small hole in the top of the can and inoculating separately with *S. aureus* and *S. marcescens*. The holes were sealed immediately and the cans incubated at 35°C for 10 days. Other cans were inoculated with a mixture of *E. coli* and a *Proteus* sp., both of which gave negative results with the TECRA Kit, together with either *S. aureus* or *S. marcescens*, in a 1:1:1 ratio. The cans were sealed immediately, incubated at 35°C for 10 days and examined for SET using the TECRA Kit.

6.2. Results and Discussion

Mushrooms which had been deliberately contaminated with SET-positive *S. aureus* or SET during cultivation were divided into two groups, one with cut and the other with uncut roots. All the samples examined tested negative with the TECRA Kit showing that SET was not absorbed or enriched even if the mushrooms were grown on SET contaminated substrates.

The levels of *S. aureus* and SET in cultivated mushroom material during an 11-day period following inoculation are shown in Figure 1. Cell counts declined sharply after the first day and no viable bacteria were detectable after day 9. Tests for SET using the TECRA Kit were negative at the end of the 11th day. The strain of *S. aureus* grown in broth for 12 h gave a positive result with the TECRA Kit showing that the experimental strain was reliable and working. These data suggest that the conditions in the cultivated material are not suitable for the growth of *S. aureus* and even less suitable for SET production.

The survival of *S. aureus* and SET production in freshly picked mushrooms is shown in Figure 2. Cell counts declined from 1.7×10^7 cells/g to 3.0×10^5 cells/g during the first 12 hours and then began to slowly increase, while the tests for SET using the TECRA Kit were negative after 42 h. Since the 42 h experimental period is way beyond the processing specifications, which stipulate that the time for mushroom collection and transportation to the canneries will not exceed 6-8 h, it is unreasonable to believe that the freshly picked mushrooms will become contaminated with SET under the present processing procedures adopted by mushroom canneries in China.

In the case of half-processed (i.e. after blanching, before retortion) mushroom products, Table 3 shows that all tests for the detection of SET, for a period of 32 h from the time of inoculation, were negative. Processing specifications state that the time from bleaching to can filling and seaming must not exceed 2.5 hr, the work benches must be clean, and workers must wash their hands at 2 hr intervals. Therefore, even if *S. aureus* contamination occurred occasionally, the bacterium does not have enough time to multiply and produce SET. The half-processed products would not be contaminated with SET under the present processing procedures adopted by mushroom canneries in China.

All SET tests conducted on damaged cans soaked in a suspension of SET-producing *S. aureus* were negative. This result suggests that the bacterium does not readily survive and produce SET in

TABLE 3. Test results for SET in contaminated half-processed products.

Time (hours)	0	4	8	12	16	20	24	28	32
SET Test	-	-	-	-	-	-	-	-	-
OD Values	0.075	0.076	0.066	0.074	0.072	0.076	0.074	0.075	0.074

Negative: OD value <0.2; positive: OD value >0.2.

canned mushrooms as a result of can defects. In actual situations, can defects may well give rise to microbial contamination although post-process contamination solely by *S. aureus* seems unlikely (English & York, 1990). Therefore, multi-species contamination was introduced in order to imitate more closely the actual situation (Table 4). When pure cultures of *S. aureus* or *S. marcescens* were introduced into cans of mushrooms through small holes drilled into the cans, and the cans incubated at 35°C for 10 days, tests for SET carried out with the TECRA Kit were positive. When *S. aureus* was inoculated together with *E. coli* and a *Proteus* sp., the can was swollen but the SET test was negative. However, when *S. marcescens* was inoculated along with *E. coli* and a *Proteus* sp., the can was again swollen and the SET test proved positive.

Although it is theoretically possible for *S. aureus* to produce SET in canned mushrooms, in reality, any can defects will lead to contamination by more than one microorganism. Under these conditions, *S. aureus* may not compete very well with the other contaminants and, under such circumstances, may not readily produce SET (English & York, 1990). Therefore, it is unlikely that the canned mushrooms will be contaminated with SET.

It should be noted that, even in the absence of *S. aureus*, other organisms in the swollen, contaminated can may cross-react with the TECRA Kit and give a 'positive' SET result.

7. CONCLUSIONS

1. Two major types of contamination of mushrooms and canned mushrooms are filth and microbial contamination. Adoption of "Good Manufacture Practice" (GMP), and "Hazard Analysis Critical Control Point" (HACCP) represent efficient measures to prevent these forms of contamination.
2. Errors can arise when the TECRA Kit is used to detect SET in Chinese canned mushrooms due to non-specific reactions shown by this kit.

Table 4. Detection of SET in canned mushrooms.

Strain	Condition of Can	TECRA Kit
Staphylococcus aureus	Flat	+
Serratia marcescens	Swollen	+
S.a + E + P	Swollen	-
S.m + E + P	Swollen	+

S.a: *Staphylococcus aureus*; S.m, *Serratia marcescens*; E: *Escherichia coli*; P: *Proteus*.

3. Experimental evidence indicates that there is no SET contamination of canned mushrooms based on the present Production Technology Procedure in China.
4. From now on, it should not be necessary to conduct SET testing of fresh and canned mushrooms as a conventional testing item.

REFERENCES

ANONYMOUS (1990). Staphylococcal enterotoxin visual immunoassay for the detection of SET A, B, C_1, C_2, C_a, D, E in food ingredients. *TECRA, Australia, Instruction booklet.*

BOMMELL (1990). SET-EIA Kit for staphylococcal enterotoxin detection. *Toxin Technology, American, Instruction booklet.*

DROUET (1991). Detection of staphylococcal enterotoxins. *TRANSIA, France, Instruction booklet.*

ENGLISH, P.H. & YORK, G. (1990). Staphylococcal food poisoning outbreaks caused by canned mushrooms from China. *Food Technology* Dec.,74-76.

PARK, CE., AKHTAR, M. & RAYMAN, M.K. (1992). Nonspecific reactions of a commercial enzyme-linked immunosorbent assay kit (TECRA) for detection of staphylococcal enterotoxins in foods. *Applied and Environmental Microbiology* **58**, 2509-2512.

SHINGAKI *et al.* (1990). SET-RPLA. A kit for the detection of staphylococcal enterotoxin A, B, C, in food samples or culture filtrates by reversed passive latex agglutination. *Oxoid, England, Instruction booklet.*

WU, Z.L., ZHANG, Z.C. & LI, C.W. (1992). Study on the nonspecific reaction problems in the procedure of using TECRA Kit (Bioenterprise Pty. Co. Australia) for detection of staphylococcal enterotoxins. *Journal of the Association for the Development of Rapid Methods and Automation in Microbiology* **4**, 105-109.