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Note

Assessment of the Microbial Contribution to the Processing of Salted Salmon Roe (*Sujiko*)

TATSURO MIYAJI*, TOMOYUKI NAKAGAWA, AND NOBORU TOMIZUKA

Department of Food Science and Technology,
Faculty of Bioindustry, Tokyo University of Agriculture,
196, Yasaka, Abashiri, Hokkaido 099-2493, Japan

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As the microbial contributions to the processing of salted foods have been little investigated, there remains a possibility that excess sterilization of raw materials for salted foods leads to deterioration in food quality and safety. At a salmon roe (*sujiko*) processing company, we investigated salted *sujiko* made identically to commercial products, but that had been processed with or without antibiotics. The antibiotics caused no significant difference in the content of free amino acids, lactic acid or acetic acid. These results show that general aerobic bacteria have no impact on the formation of these flavor compounds.

Key words : Salmon roe / Ripeness / Flavor compound / Antibiotic / Bacteria.

Salted salmon roe is a popular seafood in Japan, and there are two types of salted salmon roe, *sujiko* and *ikura*. *Sujiko* is the salted whole salmon ovary, and *ikura* is salted salmon roe in a granular form after the removal of the ovarian membrane. The major difference in the processing between the two types is the processing period. In general, the processing periods for *sujiko* and *ikura* are about seven days and one or two days, respectively. This is why the strength of flavor and bacterial number is greater for *sujiko* than for *ikura* (Kawakami et al., 2002). Therefore, more careful microbial control for *sujiko* is needed than for other salted foods that have a shorter processing time such as *ikura*. In the present report, we investigated the microbial contribution to the formation of flavor compounds, such as free amino acids, lactic acid, and acetic acid. This is the first report on microbial counts and flavor compounds during the processing of *sujiko*.

Sujiko (final conc. ca. 4% sodium chloride) was processed at a *sujiko* processing company in Hokkaido, Japan, and was processed identically to commercially sold *sujiko* made at the company except for the addition of the antibiotics. In order to investigate the microbial contribution during the processing of *sujiko* just before the whole chum salmon ovaries were processed, 100ml of an antibiotic solution (2% of sodium penicillin G, 2% of streptomycin sulfate, 0.25% of chloramphenicol and 2% of cycloheximide) was sprayed evenly on the surface of 3.5kg of ovaries in a processing container. All the antibiotics were obtained from Wako Pure Chemicals Industries, Ltd., Tokyo, Japan. As a control sample, 100ml of distilled pure water was sprayed on the surface of 3.5kg of the ovaries under identical conditions. After being processed at 15℃ for seven days, the samples were frozen at -80℃ immediately until use. After the samples were melted overnight in a cold room kept at 4℃, the samples (10g) were added to 90ml of distilled pure water, and emulsified by a stomacher. The bacterial counts on standard method agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 3% sodium chloride was determined. For determination of the flavor compounds contents, the extracts were prepared by homogenizing the frozen *sujiko* (100g) with 400ml of 6%(v/v) perchloric acid in a blender. The free amino acids and the organic acids were analyzed with an amino acid analysis system (Prominence, Shimadzu corp.,

*Corresponding author. Tel : +81-152-48-3846, Fax : +81-152-48-2940.

Kyoto, Japan) and organic acid analysis system (Shimadzu corp.) respectively.

After the processing of *sujiko* without antibiotics, i.e., in commercial processing conditions, general aerobic bacteria were at higher levels than in the non-processed *sujiko* (Table 1). These results showed that microorganisms in the *sujiko* are active during the its processing. To compare these results with commercially sold *sujiko*, *sujiko* prepared at five different companies in Hokkaido was used and tested. They were purchased from the local market, at where they had been kept cold at over 0℃ in the showcase. The bacterial number of *sujiko* processed without antibiotics was similar to that in other commercial preparations and as reported by Nagasaka (1987) and Kawakami et al. (2002). From these results, the sujiko prepared without antibiotics can be used as a model of commercially sold *sujiko*. When antibiotics were added to *sujiko* just before being processed no general aerobic bacteria was detected in the processed *sujiko* at all. This shows that sterilization of *sujiko* by spraying an antibiotic solution was sufficiently effective and there was no microbial influence on the *sujiko* processed with antibiotics.

The Japanese salted sea food *shiokara*, from squid, is processed for a long period (ca. 10 - 20 days). It is classified as a fermented food because the microbial formation of the flavor compounds, e.g., lactic acid and acetic acid has been detected during the processing of the squid *shiokara* (10% sodium chloride) at 20℃ (Fujii et al., 1999). Furthermore, *Staphylococcus* spp. is the dominant species in the bacterial flora of squid *shiokara* (Fujii et al., 1994) and *Staphylococcus* sp. St-1 and St-2 accumulated lactic acid and acetic acid in the heated extracts of squid *shiokara* (Fujii et al., 1999). In spite of the differences in the final concentration of sodium chloride in and the processing temperature for *sujiko* and squid *shiokara*, the microbial counts (ca. 10^4cfu/g) at seven days of processing and the dominant species (Kawakami et al., 2002) in the microflora were similar to each other. Moreover, Kawakami et al. (2002) reported that *Staphylococcus* spp. isolated from the commercial *sujiko* were able to form lactic acid. For these reasons, microbes might play a positive role during the processing of *sujiko* as well as squid *shiokara*. In other salted foods, a positive contribution from bacteria for the development of the ripening flavor in cured pork loins has been reported (Yamanaka et al., 2005).

We investigated the microbial contribution towards the formation of flavor compounds. Free amino acid content in the non-processed *sujiko* was similar to that in whole chum salmon ovary (Sasaki, 1994). These contents were also similar to amino acid content in chum salmon roe after removing the ovarian membrane (Suzuki and Suyama, 1983) and those in commercial *ikura* processed from chum salmon (Hayashi et al., 1990) (Table 2). As far as we know, there have been no previous reports on the content of flavor compounds such as free amino acids, lactic acid and acetic acid in *sujiko*. After the processing of *sujiko* without antibiotics, free amino acid content generally increased. In particular, L-aspartic acid, L-serine and L-glutamic acid levels significantly increased. The free amino acid content of *sujiko* processed with antibiotics was very similar to those of the *sujiko* processed without antibiotics. These results show that the formation of free amino acids dur-

TABLE 1. Bacterial counts in *sujiko*.

	Viable counts (cfu/g)
non-processed[a]	<10
processed without antibiotics	1.3×10^4
processed with antibiotics	<10
commercial *sujiko*[b]	4.4×10^4

The conditions for *Sujiko* processing and for the assay of microbial counts are described in the report. The results are mean values from three different samples from independent ovaries processed under the same conditions.
[a] the sample just before being processed.
[b] average of five preparations.

TABLE 2. Changes in free amino acid content (mg/100g) during the processing of *Sujiko* with/without antibiotics.

	Non-processed[a]	Processed	
		without antibiotics	with antibiotics
L-Asp	13.8±0.5	23.9±1.3	29.5±7.3
L-Thr	6.6±0.1	14.9±2.9	12.6±0.5
L-Ser	9.7±0.5	39.5±2.9	43.7±4.9
L-Glu	16.9±0.6	31.8±2.5	32.3±3.9
L-Pro	1.5±0.1	4.7±0.3	4.8±0.2
Gly	1.7±0.1	4.9±0.2	4.7±0.2
L-Ala	4.1±0.3	12.2±0.6	11.7±0.7
L-Cys	3.1±0.2	4.9±0.5	3.8±0.7
L-Val	7.4±0.6	14.2±0.7	14.2±0.3
L-Met	3.6±0.1	6.2±0.3	5.9±0.2
L-Ile	5.1±0.1	11.6±0.5	11.4±0.6
L-Leu	8.0±0.2	15.7±0.7	14.7±0.3
L-Tyr	3.7±0.2	6.0±0.2	5.7±0.4
L-Phe	8.3±0.3	12.7±0.7	11.8±0.7
L-His	2.9±0.1	5.6±0.3	6.1±0.6
L-Lys	6.8±0.2	18.1±1.2	17.9±1.1
L-Arg	4.2±0.1	12.3±0.9	12.7±0.8

The conditions for the assay of free amino acids contents are described in the text. The results are mean values ± SD from three samples from different ovaries processed under the same conditions.
[a] the sample just before being processed.

TABLE 3. Changes in organic acid content (mg/100g) during the processing of *Sujiko* with/without antibiotics.

	Non-processed[a]	Processed	
		without antibiotics	with antibiotics
Lactic acid	4.09±0.43	6.97±0.66	7.74±1.35
Acetic acid	3.39±0.35	2.53±0.38	1.79±0.2

The conditions for the assay of organic acid contents are described in the text. The results are mean values ±SD from three samples from different ovaries processed under the same conditions.

[a] the sample just before being processed.

ing the processing is due to autolysis, and not due to the microorganisms. These results are identical to those reported for squid *shiokara* (Fujii et al., 1994).

Lactic acid content increased and acetic acid content decreased after processing of *sujiko*. Contrary to our expectation, there was no difference in the levels of these two compounds processed with or without antibiotics (Table 3). Furthermore, although several unknown organic acids were detected in *sujiko*, there was no difference in their content of the *sujiko* processed with antibiotics and without them (data not shown). This indicates that organic acid content during the processing is not associated with microbial activity, but is caused by intracellular reactions following autolysis. These results are different from those reported on squid *shiokara* (Fujii et al., 1999). The difference in the changes of organic acid content in *sujiko* and squid *shiokara* may be attributed to different bacterial flora between them. It may be possible that *sujiko* is at an earlier stage of change in the bacterial flora than squid *shiokara*.

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