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A Research Note

Evaluation of the Bacteriological Safety of Low-Salt Miso

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ABSTRACT

Studies were done to evaluate the safety of three different low-salt (2.36 to 5.79% NaCl) misos inoculated with different bacterial pathogens. *Clostridium botulinum* types A and B (inoculum level of ca. 120 spores/g) did not produce toxin in any of the misos within 18 wk at 25°C. *Staphylococcus aureus, Salmonella typhimurium* and *Yersinia enterocolitica* (inoculum level of ca. 10^3 to 10^4 CFU/g) progessively died in all of the misos held at either 10 or 25°C. The miso samples, which were obtained from Japan (3.75 and 5.79% NaCl) and California (2.36% NaCl), had water activities of 0.843, 0.835 and 0.875, respectively, and pH values of 5.26, 5.30 and 4.73, respectively. Results indicate that low-salt misos with these properties are not likely to be bacteriological health risks.

Miso is a traditional oriental seasoning that is used extensively in Japan, and to a lesser extent in China and other parts of the Orient (*1*). In recent years, miso has become increasingly popular in the United States. In its original form, miso is a high-salt, fermented food, having a sodium chloride content ranging from 8 to 14% (*2*). The product is made from rice, barley, soybeans, and sometimes wheat which are fermented by a series of microorganisms, including molds (such as *Aspergillus oryzae*), yeasts (such as *Saccharomyces* sp.), and lactic acid-forming bacteria (such as *Pediococcus halophilus* or *Streptococcus faecalis*) (*1,2*). Salt is added to serve as a preservative and to exert a selective action on the microorganisms which grow during the fermentation (*1*). In addition to its high salt content, miso contains large amounts of amino acids and sometimes ethanol, which in combination with salt often reduce the water activity (a_w) of miso to levels of 0.85 or lower (*6*).

Traditionally, miso has been a safe product. However, low-salt products are becoming increasingly popular, and misos containing reduced levels of sodium chloride are being marketed. Considering that miso may be held for months at room temperature, an evaluation of microbiological safety of low-salt misos was deemed necessary. The purpose of this study was to determine the survival and growth or toxin-producing abilities of four bacterial pathogens in different preparations of low-salt miso.

MATERIALS AND METHODS

Bacterial cultures and methods of enumeration

Four foodborne bacterial pathogens were studied. These included: *Clostridium botulinum, Staphylococcus aureus, Salmonella typhimurium* and *Yersinia enterocolitica*. Methods for their growth, for preparation of inocula, for enumeration, and for detection of toxin have been described previously (*5,10*).

Miso

Low-salt misos produced in Japan (Japan A, 3.75% NaCl and Japan B, 5.79% NaCl) were obtained from Nagano Miso, Inc., Ueda-Shi, Japan. They were shipped to our laboratory in sealed aluminum pouches and were kept at 4°C until used. Sweet red (Edo) miso (California, 2.36% NaCl) was produced by GEM Cultures, Fort Bragg, CA, according to the procedure described by Shurtleff and Aoyagi (*8*). The fermentation was at 32°C for 32 d. This preparation was shipped to our laboratory by Federal Express and was stored at 4°C until used.

Inoculation and incubation of miso

A bacterial inoculum was prepared as reported previously (*5*). The inoculum was added dropwise (5 ml per kg) and mixed with miso using a Kitchenaid food mixer (Model KS-A, Hobart Corp., Troy, OH). The inoculated product (10 g/tube) was packed using a grease-gun in 17 × 100-mm sterile, disposable polystyrene tubes with polyethylene closures. *C. botulinum* inoculated samples were incubated anaerobically in GasPak jars. Other samples were incubated in plastic tubes that were capped tightly with plastic caps.

Analyses

pH. Determination of pH was done using an Altex (Model 3560, Beckman Instruments, Irvine, CA) digital pH meter equipped with a Futura combination electrode that was immersed directly into the miso. Standardization of the pH meter was done with pH 7.00 and 4.00 buffers (Fisher Scientific Co., Chicago, IL).

Chloride. Determination of chloride was done using the silver nitrate-thiocynate method described in the *Official Methods of Analysis (3)*.

Water activity (a_w). Determination of a_w was done using Hygroline sensors with a recording device (Model VFB, Beckman Instruments, Cedar Grove, NJ). The instrument was calibrated using different concentrations of sodium chloride solutions (7, Tanaka, unpublished data). To eliminate the influence of ethanol that may be present in miso, a carbon-impregnated filter (Beckman Instruments) was used in the sensor housing.

RESULTS

The three miso samples evaluated had low salt content (2.36 to 5.79% NaCl), acidic pH (pH 4.73 to 5.30), and intermediate water activity (a_w 0.835 to 0.875) (Table 1).

TABLE 1. *Analysis of miso*[a].

Miso	pH	a_w[b]	NaCl (%)
Japan A	5.26	0.843	3.75
Japan B	5.30	0.835	5.79
California	4.73	0.875	2.36

[a] Each value is an average of duplicate determinations.
[b] a_w was measured using a Beckman Hygroline apparatus with sodium chloride solutions as standards.

C. botulinum did not produce toxin in any of the three miso samples held at 10 or 25°C for as long as 18 wk of incubation (data not shown).

S. aureus (Table 2), *S. typhimurium* (Table 3) and *Y. enterocolitica* (Table 4) progressively died during storage in all the miso samples tested, although there were some differences in death rates from one experiment to another. In general, the organisms died more rapidly when held at 25°C than at 10°C.

DISCUSSION

None of the pathogens grew in any of the low-salt miso samples, with *S. aureus, S. typhimurium* and *Y. enterocolitica* progressively dying during extended storage.

Even though the miso samples had low levels of salt, they also had relatively low a_w values. Miso having only 3.75 and 2.36% sodium chloride had a_w values of 0.843 and 0.875, respectively. The low a_w was likely due to a combined effect of the sodium chloride and the high concentrations of amino acids and peptides, and possibly ethanol, produced during the fermentation of miso (*2*). This low a_w should completely inhibit the growth of *C. botulinum (4,9)* and *Salmonella* spp. (*9*), and retard or prevent the growth of *S. aureus (9,11,12)*.

TABLE 2. *Survival of* Staphylococcus aureus *in miso*[a].

Experiment No.	Miso	Temperature (°C)	Log_{10} *S. aureus*/g at day: 0	1	2	3	7
I	Japan A	10	3.79	1.41	-[b]	0.30	-
	Japan B	10	4.86	3.98	-	3.49	-
II	Japan A	10	3.77	3.00	2.76	-	1.90
		25	3.77	2.59	1.48	-	0.84
	California	10	3.19	2.67	2.53	-	1.39
		25	3.19	2.52	1.67	-	0.30

[a]Each value is an average of triplicate determinations.
[b]-, not tested.

TABLE 3. *Survival of* Salmonella typhimurium *in miso*[a].

Experiment No.	Miso	Temperature (°C)	Log_{10} *S. typhimurium*/g at day: 0	1	2	7
I	Japan A	10	3.08	0.30	-[b]	-
	Japan B	10	4.08	0.30	-	-
II	Japan A	10	3.66	3.34	3.09	1.48
		25	3.66	1.60	2.08	0.75
	California	10	3.82	3.35	3.33	1.59
		25	3.82	2.72	1.85	0.30

[a]Each value is an average of triplicate determinations.
[b]-, not tested.

TABLE 4. *Survival of* Yersinia enterocolitica *in miso*[a].

Miso	Temperature (°C)	Log_{10} *Y. enterocolitica*/g at day: 0	1	2	7
Japan A	10	3.57	3.36	2.49	1.69
	25	3.57	2.56	2.52	0.86
Japan B	10	3.00	2.25	1.94	<0.30
	25	3.00	2.49	2.30	<0.30
California	10	3.71	3.24	2.75	1.68
	25	3.71	2.60	2.20	0.86

[a]Each value is an average of triplicate determinations.

Furthermore, the pH levels of miso samples were relatively low (Table 1). The combined effect of low pH and low a_w levels was likely responsible for the growth inhibitory and, in many instances, lethal effect of low-salt miso on bacterial pathogens. Not considered as part of this study yet a potential concern, is the possible growth and toxin production by toxigenic molds on low-salt miso.

Although there were differences in death rates between experiments, generally pathogens were more rapidly inactivated in miso at 25°C than at 10°C. A similar observation was reported by Kubota et al. (*6*), who evaluated the survival and growth characteristics of pathogenic *Escherichia coli, S. aureus* and *Vibrio parahaemolyticus* in miso with 0, 6.4 or 11.2% sodium chloride. Kubota et al. (*6*) found that the bactericidal action of miso, including preparations with 0% NaCl, was greater as the incubation temperature was increased (30°C > room temperature > 5°C). They also found that the a_w values of their low-salt miso samples (i.e., a_w of 0.905 for 0% NaCl miso and a_w of 0.828 for 6.4% NaCl miso) were in the same range as the samples we evaluated (Table 1). Of significance, the miso samples that Kubota et al. (*6*) evaluated contained about 4% ethanol, which they indicated was produced during the fermentation (we did not assay our samples for ethanol).

Results of our studies and those of Kubota et al. (*6*) suggest that low-salt miso having relatively low a_w and pH values, is not a bacteriological health hazard.

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