

Heat Treatment for Elimination of Red-Halophiles from Contaminated Solar Salt

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In an effort to develop a suitable method for prevention of red-discolouration in cured fish, the efficacy of heat treatment was evaluated in eliminating red halophiles from contaminated salt samples. Naturally contaminated salt samples containing red halophiles as well as sterilized salt samples artificially contaminated with red-halophilic Cocci were employed in this study. Heating of contaminated salt at a temperature of 80°C for 30 min, was found to be effective in eliminating red-halophilic Cocci.

Key words : Red-discolouration, cured fish, heat treatment, solar salt

Red discolouration of sea water upon evaporation was described in ancient Chinese treatise written about 2700 BC (Beatty & Fougere, 1957). This is because of extreme halophilic bacteria, which are involved in spoilage of salted fish. Different studies have shown the extent of spoilage caused by red-halophilic bacteria in salt cured fish (Rao *et al.*, 1962, Joseph *et al.*, 1983, 1988). During storage these bacteria grow on salt cured fish and the spoilage takes place in a few weeks (Wibowo *et al.*, 1990; Prasad & Rao, 1994).

Earlier studies indicated destruction of red-halophiles in solar salt by dry heat although they could be more easily killed by steam under pressure than the dry heat treatment (Castell, 1950). Kushner *et al.* (1965) have described a simple method of inactivation of halophilic bacteria in contaminated solar salt using acetic acid or hydrochloric acid.

The present study was undertaken to evaluate the methods of heat treatment for elimination of red-halophilic bacteria from contaminated salt.

Materials and Methods

Solar salt samples less than a month old were collected from manufacturing premises and market in sterile containers. Total red halophilic bacterial counts were determined by diluting 10 g salt samples in 90 ml sterile saline (20% NaCl w/v). Serial dilutions were prepared by adding one ml of this to 9 ml of the same diluent. Skim milk salt agar (Sreenivasan & Venkataraman, 1956) was employed for isolation of red-halophilic bacteria. Inoculated plates were incubated at 37±1°C for 4 weeks. Plates were kept in sealed polythene bags to avoid dehydration.

Heat treatment was carried out on both naturally and artificially contaminated salt samples. For artificial contamination, salt samples were sterilised by heating for 2 h at 160°C in a hot air oven followed by autoclaving at 121°C for 20 min. Three day old cultures of *Halococcus* spp. in halophilic broth incubated at 37°C were harvested by centrifugation at 5000 rpm for 30 min and washed twice in sterile saline. The washed pellets were diluted with same diluent and thoroughly mixed to yield a final halophilic count of 10^6 - 10^7 cfu g⁻¹.

For heat treatment, salt samples were spread uniformly in 2.5 cm thick layers in a hot air oven equipped with built-in thermometers. Temperatures of the salt were recorded at regular intervals at the bottom and top of the salt layers at several places and the average taken for each observation. Oven temperatures were recorded from built-in thermometers. Samples were withdrawn at regular intervals and red halophilic counts and moisture content (AOAC, 1980) were determined. Incubation period for red halophilic counts were extended from one month during preliminary trials to 4 months in subsequent trials.

Naturally contaminated salt samples were heat treated by (i) keeping in oven at ambient temperature and heating to 100°C; (ii) keeping in pre-heated oven (150°C) and then raising the temperature to 171°C; and (iii) keeping in oven at ambient temperature and heating to 150°C. Artificially contaminated salt samples were heat treated by (i) keeping in a preheated oven maintained at 150°C up to 40 min and (ii) heating the salt at 80°C for 30 min.

Results and Discussion

Naturally contaminated salt from salt pans showed initial counts of 4.0×10^4 and 5.6×10^4 cfu g⁻¹ of red halophilic bacteria. When these salt samples were heated from ambient to 100°C, the salt reached a temperature of 58°C after 30 min in both cases. Although the heating was continued for a further 30 min, consistent results were not obtained. This was traced to inadequacy of the temperature of heating and length of incubation period for the survivors. Subsequently higher temperatures were used for heating and an incubation period of 4 months was adopted.

Results of subsequent trials with naturally contaminated solar salt are shown in

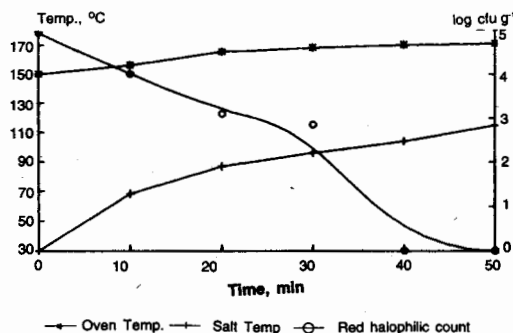


Fig. 1. Effect of heat treatment on red-halophile contaminated solar salt kept in pre-heated oven (150°C) and raising oven temperature to 171°C

Figs. 1, 2 & 4, when salt was kept in a preheated oven (150°C) which was heated further. Temperature equilibrium was not attained between oven and salt, even after 50 min, over which the oven temperature was raised to 171°C. The salt temperature was only 115°C indicating a poor heat transfer rate. In the second trial, when the salt and oven were heated from ambient temperature to 150°C (Fig. 2), similar conditions prevailed. While it took 40 min for the complete elimination of red halophiles in the former trial, it took longer (75 min) in the latter, owing to the lower initial temperature and slower rate of heating. However, in both cases the highest destruction of red halophiles occurred in the temperature range of 90-104°C and moisture level of less than 1 percent (Fig. 4).

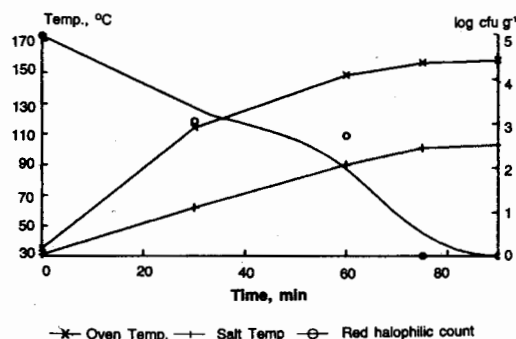


Fig. 2. Effect of heat treatment on red-halophile contaminated solar salt kept in oven at ambient temperature and heating to 150°C

Table 1. Effect of heat treatment on artificially contaminated solar salt samples

Trial	Moisture in salt, %		Initial red halophilic count, cfu g ⁻¹ salt	Oven temperature, °C	Salt temperature, °C	Duration of heating, min	Final red halophilic count cfu g ⁻¹ of salt
	Initial	Final					
1	8.03	3.10	2.55x10 ⁷	150	78	15	5.59x10 ⁴
2	6.65	1.31	1.15x10 ⁷	150	80	20	2.50x10 ²
3	9.80	0.80	2.73x10 ⁷	150	75	40	ND
4-7*	7.00	0.65	3.40x10 ⁶	-	80	30	ND

* average values; ND - Not detected

Based on these trials, a constant oven temperature of 150°C was adopted for treating the artificially contaminated salt samples, the results of which are depicted in Figs. 3 & 4 and Table 1. The minimum period of treatment was observed to be 40 min for an initial contamination level of 8.63 log cfu g⁻¹. The highest destruction of red halophiles occurred at 67-73°C between 15 and 20 min of heating. The survivor counts showed a broken curve behaviour. A high rate of destruction in the initial 10 min of heating (corresponding to a decimal reduction time of 2.45-4.2 min) later gave way to a more moderate rate of destruction (decimal reduction time: 7-10 min) between 20-40 min of heating when the salt reached a near constant temperature of 70-75°C.

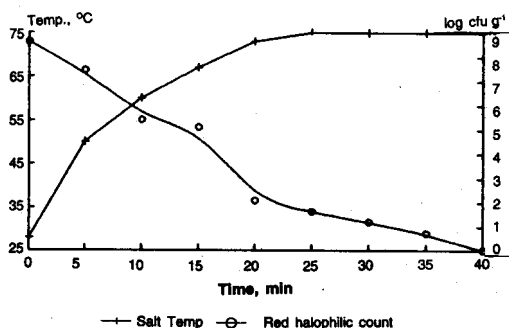


Fig. 3. Effect of heat treatment on solar salt artificially contaminated with red halophilic cocci kept in pre-heated oven (150°C) and maintained at 150°C

A point of similarity between heating of naturally and artificially contaminated salt samples was that inspite of differing levels of contamination, both took roughly the same time (about 30-40 min) for total elimination of red halophiles within a temperature range of 68-104°C (Av.: 86°C) for the former and 75°C for the latter.

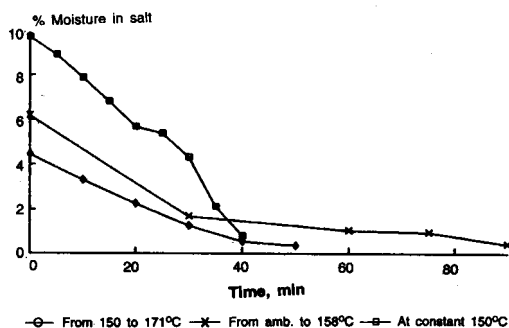


Fig. 4. Effect of different heat treatments on moisture level of solar salt

Subsequent trials confirmed (Trials 4-7; Table 1) that an initial contamination level of 3.4x10⁶ g⁻¹ could be fully overcome by treating the artificially contaminated salt at 80°C for 30 min.

On the basis of foregoing observations, it can be said that heating salt at a temperature of 80°C for 30 min eliminates red halophilic cocci, which are mostly responsible for red-discolouration in salt cured fish. But in naturally contaminated

salt, both red halophilic rods and cocci may occur, when the proposed heat treatment could prove inadequate. Differences in survival of red halophiles between heated salt samples could be due to difference in their flora and moisture content. Protective action of salt crystals on red halophiles has been proposed by Lamprecht & Riley (1990). Further studies are need to scale up the results for commercial quantities of salt.

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