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Effect of Potassium Sorbate and Sodium Benzoate on *Listeria monocytogenes* in Freshwater Fish, Prawn and Chicken Meat

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ABSTRACT

Listeria monocytogenes causes several illnesses in human beings and animals in the form of both outbreaks and sporadic infections. Infections due to *L. monocytogenes* are mostly acquired through consumption of different kinds of foods including both raw and processed foods. In the study, combination of two preservatives viz., sodium benzoate and potassium sorbate was found very effective in controlling the growth of *L. monocytogenes* in freshwater fish and chicken meat and it was more pronounced in freshwater fish than chicken meat. Combination of these two preservatives at 0.1% level was found much effective in freshwater fish, whereas combination at 0.2% level was required in case of chicken meat in controlling growth of *L. monocytogenes*.

Keywords: Benzoate, chicken, fish, *Listeria*, prawn, sorbate

Introduction

Listeria monocytogenes is a potential human and animal pathogen causing many serious illnesses which include abortion, infertility, arthritis, gastroenteritis, septicemia, meningitis, conjunctivitis etc. (Aureli *et al.*, 2000; Va´zquez-Boland *et al.*, 2001). The organism is also responsible for specific nervous system disorder called 'circling calf syndrome' in ruminants (Vishwanathan and Ayyar, 1950). Generally, human being acquires listeria infection mostly through consumption of different food items contaminated with *L. monocytogenes* leading to both outbreaks and sporadic cases (Jacquet *et al.*, 1995; Dalton *et al.*, 1997). The ability of this pathogen to grow at refrigerated temperature is the reason for serious public health hazards (Junttila *et al.*, 1988).

Listeria infections have also been reported in human beings due to consumption of different fish and fish products (Riedo *et al.*, 1994; Ericsson *et al.*, 1997). There are also the reports of incidence of outbreak of *L. monocytogenes* in poultry and thus there is possibility of transmission of listeriosis through poultry products (Nagi and Verma, 1967). So, the control of growth of *L. monocytogenes* in different food products is of utmost important. Potassium sorbate and sodium benzoate are two commonly used preservatives in food and the effect of these two preservatives against different pathogens have been studied by some workers (Beuchat, 1980; Hazarika *et al.*, 2003).

In the present study, the sodium benzoate and potassium sorbate was studied for their

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ability to check the growth of *Listeria monocytogenes* in fish, prawn and chicken homogenates.

Materials and Methods

Freshwater fish 'Rohu' (*Labeo rohita*) and chicken meat used in this study were procured from the local markets of Bareilly, (Uttar Pradesh,) whereas giant fresh water prawn (*Macrobrachium rosenbergii*) was procured from INA market complex, Delhi.

Preparation of fish, prawn and chicken homogenates

The homogenates from fish, prawn and chicken meat were prepared as per the method of Matches *et al.* (1971) with slight modifications. One part of respective meat was blended in four parts of normal saline in a stomacher blender till tissues were thoroughly broken down. Then it was autoclaved, cooled, adjusted to pH 7.0 and again blended. The homogenates were held in flowing steam for 15 min to inactivate any contaminations that might have gained entrance during pipetting and then 10 ml of homogenate was distributed in each tube. The stock solutions of 5% potassium sorbate and 5% sodium benzoate were prepared separately and sterilized by filtration through Millipore filter (0.22 µ). The required amount of these solutions were added to each tube to make homogenate with 0.1% and 0.2% each of potassium sorbate and sodium benzoate along with their combinations in case of each fish, prawn and chicken homogenates, separately, along with the controls.

Organism

Listeria monocytogenes (MTCC 657) used for spiking was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The strain was maintained on tryptic soya agar slope at 4°C and characterized and confirmed by Gram's reaction and standard biochemical reactions (Lovett, 1987).

Artificial spiking

The loopful of culture of *L. monocytogenes* was inoculated into BHI broth and incubated at 37°C for 24 h. A 100 µl from 10^{-4} diluted 24 h grown culture of *L. monocytogenes* was added to each tube containing different levels of these two preservatives. After spiking, the plating was done for enumeration of initial population (0 h) and then incubated at 37°C. Counting of *Listeria* population was carried out by standard plate count method at 12, 24, 36 and 48 h on tryiptic soya agar along with 0.6% yeast extract (TSAYE).

Results and Discussion

Potassium sorbate alone was not found much effective in any concentration in checking the growth of *L. monocytogenes* in any homogenate of fish, prawn and chicken meat, while sodium benzoate was found little effective at 0.1% level. However, moderate effect was recorded on the growth of the organism at 0.2% level in fish and chicken. In fish homogenate, 0.2% sodium benzoate had inhibited the growth of *L. monocytogenes* by almost 4.4 $\log_{10}$ in comparison to the control (4.217 against 8.685) as shown in Table 1. Approximately, 1.8 $\log_{10}$ and 4.8 $\log_{10}$ reduction in growth was obtained in chicken meat as compared to control on using 0.1% and 0.2% sodium benzoate, respectively (Table 2). However, in case of prawn, neither potassium sorbate nor sodium benzoate was found effective to inhibit the growth of *L. monocytogenes* (Table 3). Combination of potassium sorbate and sodium benzoate at 0.1% level was found moderately effective against the organism in fish homogenate as it inhibited the growth by almost 4 $\log_{10}$ as compared to control (4.653 against 8.685), while approximately 3 $\log_{10}$ inhibition was in chicken meat. On using the concentration of 0.2% potassium sorbate and sodium benzoate in combination, the growth of the organism was inhibited to a great extent in

Table 1: Effect of potassium sorbate and sodium benzoate on the growth of *L. monocytogenes* in homogenate of Fresh Water

Preservatives	*Population of *L. monocytogenes* at different periods (Hours)				
	0	12	24	36	48
0.1% Potassium sorbate	3.978	6.720	7.961	8.550	8.618
0.2% Potassium sorbate	4.021	5.712	7.415	8.439	8.465
0.1% Sodium benzoate	4.217	5.845	6.914	7.934	8.398
0.2% Sodium benzoate	4.021	4.724	4.782	4.799	4.217
Combination of 0.1% potassium sorbate and 0.1% sodium benzoate	4.025	4.525	4.623	4.681	4.653
Combination of 0.2% potassium sorbate and 0.2% sodium benzoate	4.021	4.176	3.854	3.389	3.415
Control	4.004	6.959	8.623	8.662	8.685

*In $\log_{10}$ CFU/ml (Mean value of two observations)

Table 2: Effect of potassium sorbate and sodium benzoate on the growth of *L. monocytogenes* in homogenate of chicken meat

Preservatives	*Population of *L. monocytogenes* at different periods (Hours)				
	0	12	24	36	48
0.1% Potassium sorbate	3.732	7.916	8.964	9.043	9.568
0.2% Potassium sorbate	3.785	7.895	8.884	8.439	9.512
0.1% Sodium benzoate	3.716	5.857	6.217	7.505	8.556
0.2% Sodium benzoate	3.861	3.623	4.398	4.857	5.550
Combination of 0.1% potassium sorbate and 0.1% sodium benzoate	3.699	3.362	4.607	6.792	7.061
Combination of 0.2% potassium sorbate and 0.2% sodium benzoate	3.712	3.041	2.332	3.792	4.903
Control	3.732	8.914	9.431	10.079	10.322

*In $\log_{10}$ CFU/ml (Mean value of two observations)

case of fish and chicken homogenate as it decreased the listerial population from 4.021 to 3.415 $\log_{10}$ CFU/ml in fish homogenate at 48 h and very marginally increased from 3.712 to 4.903 $\log_{10}$ CFU/ml in chicken meat, but no effect was observed on the growth of the organism in case of prawn (Table 1, 2, 3).

The findings of present study indicated that the combination of potassium sorbate and sodium benzoate were found very much effective in controlling the growth of *L. monocytogenes* in fish and chicken homogenate, but not in prawn homogenate. This may be due to the different pH values of freshwater fish, prawn and chicken meat. Although once at the initial stage, pH was adjusted at 7.0, but it may have changed afterwards. As

Table 3: Effect of potassium sorbate and sodium benzoate on the growth of *L. monocytogenes* in homogenate of pawn

Preservatives	*Population of *L. monocytogenes* at different periods (Hours)				
	0	12	24	36	48
0.1% Potassium sorbate	4.352	7.322	8.653	9.049	9.139
0.2% Potassium sorbate	4.371	7.312	8.623	8.914	8.959
0.1% Sodium benzoate	4.322	7.290	8.906	9.066	9.105
0.2% Sodium benzoate	4.362	7.176	8.484	8.892	8.884
Combination of 0.1% potassium sorbate and 0.1% sodium benzoate	4.415	7.041	8.580	8.796	8.845
Combination of 0.2% potassium sorbate and 0.2% sodium benzoate	4.398	7.019	8.484	8.623	8.771
Control	4.342	9.079	9.580	10.267	10.043

* In $\log_{10}$ CFU/ml (Mean value of two observations)

per some previous reports, both the preservatives are more active in acidic pH (Padilla-Zakour, 1998). In this study, it was observed that the combination of potassium sorbate and sodium benzoate was more effective than any one of them using alone in controlling the growth of this organism. Hazarika *et al.* (2003) also reported that the combination of 0.1% potassium sorbate and sodium benzoate was effective in controlling the growth of *E. coli* VTEC O111 in simulating media for beef gravy. Combination of these preservatives at 0.1% level was effective in controlling the growth of *L. monocytogenes* in fish homogenate, while at 0.2% level in case of chicken meat. As per USFDA norms, the maximum permissible limit of potassium sorbate and sodium benzoate is 0.1% (Padilla-Zakour, 1998). So, it can be stated that combination of two preservatives may be used to check the growth of *L. monocytogenes* specifically in fish homogenate.

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