



Efficiency of chemical preservatives on the inhibition of spoilage causing bacteria in yellow goat fish (*Sulphureus cuvier*)

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Abstract

The present study was aimed to study the efficiency of various Chemical preservatives on the inhibition of spoilage causing bacteria in Yellow Goat Fish (*Sulphureus cuvier*). The effect of chemical preservatives on the inhibition of pathogenic bacteria was estimated by Agar well diffusion method. The diameter of the inhibition zone of all the bacterial cultures was increased with increase in concentrations of chemical preservatives from 200 to 1000 $\mu\text{l ml}^{-1}$. The effect of various chemical preservatives on the inhibition of the growth of bacteria isolated from spoiled fish samples was determined. All the concentrations showed inhibitory zone against all the test bacterial isolates. Sodium metasilphite exhibited a maximum zone of inhibition against *Staphylococcus aureus* followed by *Salmonella typhi*, *Pseudomonas fluorescens*, and *Vibrio cholerae*. The least zone of inhibition was observed in *Escherichia coli*. Among the pathogens tested, *Staphylococcus aureus* was found to be more susceptible to chemical preservatives and *Escherichia coli* was found to be less susceptible.

Keywords: yellow goat fish (*Sulphureus cuvier*), fish spoilage, bacterial pathogens, sodium metasilphite

Introduction

Fish is an extremely perishable food in many countries all over the universe (Ojewola and Annah, 2006) [2]. Various factors are responsible for fish spoilage. Fish spoilage is brought about mainly by, the enzymes present in the live fish. The enzymes begin to break down fish tissues. Before death, the enzymes were involved in the digestion of ingested food and all enzymatic reactions are controlled. In the dead fish, the control system fails and the enzymes begin to act on the alimentary system and fish flesh, thereby resulting in soft destructive changes (Ravichandran *et al.*, 2010) [4]. Bacteria are present in the gut, gills, and skin surfaces of live fish (Sutharshing and Sivashanthini, 2011) [5]. The live fish defense mechanism is able to combat the action of these bacteria. However, after death, this defense mechanism also fails. Consequently, the bacteria invade the gut, gills, and skin, and cause decomposition from within and on the exposed surfaces of the fish (Wefky and Ghobrial, 2008) [6]. During fish spoilage, there is a breakdown of various components and the formation of new compounds. These new compounds are responsible for the changes in odour, flavor and texture of the fish meat (Akinyemi and Buoro, 2011) [1].

Materials and Methods

1. Collection of fish samples

The spoiled fish (Yellow goat fish – *Sulphureus cuvier*) was collected from three different locations *viz.*, Chidambaram fish market, Killai and Bhuvanagiri. The collected spoiled fish samples were preserved in the refrigerator at 4 °C.

2. Effect of Chemical preservatives on the inhibition of growth of Bacterial fish pathogens

The effects of different concentrations of chemical preservatives (Sodium nitrite, Potassium nitrite, Sodium bisulphite, Sodium metasilphite, Potassium bisulphite and Potassium metasilphite) on the inhibition of bacterial strains were studied by using Agar well diffusion assay. The sterilized Nutrient agar medium was prepared and seeded with a standard inoculum of the five bacterial strains, *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas fluorescens* separately and plated. Different concentrations of chemical preservatives *viz.*, 200, 400, 600, 800, and 1000 $\mu\text{g ml}^{-1}$ were prepared. On the surface of the medium, wells were made by using cork borer (6 mm in diameter) and 100 μl of concentrations was discharged into the well. The plates were incubated at room temperature for 24 hrs and the diameter of the inhibition zone (in mm) around the well was measured. Three replications were maintained in each treatment.

Results and Discussion

1. Effect of Sodium nitrite on Fish spoilage bacteria

The effect of Sodium nitrite on the inhibition of growth of bacteria isolated from spoiled fish samples was determined and the results were furnished in Table - 1. The diameter of the inhibition zone of all the bacterial cultures was increased with increase in concentrations of Sodium nitrite from 200 to 1000 $\mu\text{g ml}^{-1}$. All the concentrations showed inhibitory zone against all the test bacterial isolates. Sodium nitrite exhibited a maximum zone of inhibition against *Staphylococcus aureus* (32 mm) followed by *Salmonella typhi* (30 mm), and *Pseudomonas fluorescens* (29 mm), and *Vibrio cholerae* (28 mm). The most minor zone of inhibition was observed in *Escherichia coli* (26 mm).

Table 1: Effect of Sodium nitrite on inhibition of Fish spoilage bacteria

Sodium nitrite ($\mu\text{g ml}^{-1}$)	Diameter of inhibition zone (in mm)				
	<i>V. cholera</i>	<i>E. coli</i>	<i>P. fluorescens</i>	<i>S. typhi</i>	<i>S. aureus</i>
200	10	10	13	15	18
400	15	12	16	17	20
600	17	15	21	23	25
800	24	21	26	27	29
1000	28	26	29	30	32

2. Effect of Potassium nitrite on Fish spoilage bacteria

The effect of Potassium nitrite on the inhibition of growth of bacteria isolated from spoiled fish was analyzed and the results were tabulated in Table - 2. The diameter of the inhibition zone of all the bacterial culture was increased with increase in concentrations of tartaric acid from 200 to 1000 $\mu\text{l ml}^{-1}$. No zone of inhibition was observed at 200 $\mu\text{g ml}^{-1}$ against *Vibrio cholerae*, *Escherichia coli* &

Pseudomonas fluorescens and at 400 $\mu\text{l ml}^{-1}$ concentration, no zone of inhibition was recorded against *Escherichia coli* and *Vibrio cholerae*. Potassium nitrite exhibited a maximum zone of inhibition against *Staphylococcus aureus* (24 mm) followed by *Salmonella typhi* (22 mm), *Pseudomonas fluorescens* (21 mm) and *Vibrio cholerae* (20 mm). The least zone of inhibition was observed in *Escherichia coli* (18 mm).

Table 2: Effect of Potassium nitrite on the inhibition of Fish spoilage bacteria

Potassium nitrite ($\mu\text{g ml}^{-1}$)	Diameter of inhibition zone (in mm)				
	<i>V. cholera</i>	<i>E. coli</i>	<i>P. fluorescens</i>	<i>S. typhi</i>	<i>S. aureus</i>
200	NZ	NZ	NZ	9	10
400	NZ	NZ	10	11	12
600	11	9	13	15	17
800	16	13	18	19	21
1000	20	18	21	22	24

NZ – No Zone of inhibition

3. Effect of Sodium metasilphite on Fish spoilage bacteria

The effect of Sodium metasilphite on the inhibition of growth of bacteria isolated from spoiled fish was studied and the results were shown in Table - 3. The diameter of the inhibition zone of all the bacterial culture was increased with increase in concentrations of Sodium metasilphite from 200 to 1000 $\mu\text{l ml}^{-1}$. The diameter of the inhibition zone of all the bacterial culture was increased with increase

in concentrations of Sodium metasilphite from 200 to 1000 $\mu\text{g ml}^{-1}$. All the concentrations showed inhibitory zone against all the test bacterial isolates. A maximum zone of inhibition was observed against *Staphylococcus aureus* (36 mm) followed by *Salmonella typhi* (35 mm), *Pseudomonas fluorescens* (33 mm) and *Vibrio cholerae* (32 mm). The most minor zone of inhibition was observed in *Escherichia coli* (30 mm).

Table 3: Effect of Sodium metasilphite on inhibition of Fish spoilage bacteria

Sodium metabisulphite ($\mu\text{g ml}^{-1}$)	Diameter of inhibition zone (in mm)				
	<i>V. cholera</i>	<i>E. coli</i>	<i>P. fluorescens</i>	<i>S. typhi</i>	<i>S. aureus</i>
200	14	13	16	18	20
400	18	15	19	20	22
600	20	18	24	26	28
800	28	25	30	32	33
1000	32	30	33	35	36

4. Effect of Potassium metasilphite on fish spoilage bacteria

The effect of Potassium metasilphite on the inhibition of growth of five fish spoilage bacteria was investigated and the results were given in Table - 4. The diameter of the inhibition zone of all the bacterial culture was increased with increase in concentrations of Potassium metasilphite from 200 to 1000 $\mu\text{g ml}^{-1}$. No zone of inhibition was

observed at 200 $\mu\text{g ml}^{-1}$ against *Vibrio cholerae* and *Escherichia coli*. Potassium metabisulphite exhibited maximum zone of inhibition against *Staphylococcus aureus* (28 mm) followed by *Salmonella typhi* (26 mm), *Pseudomonas fluorescens* (25 mm) and *Vibrio cholerae* (24 mm). The least zone of inhibition was observed in *Escherichia coli* (22 mm).

Table 4: Effect of Potassium metasilphite on the inhibition of fish spoilage bacteria

Potassium metabisulphite ($\mu\text{g ml}^{-1}$)	Diameter of inhibition zone (in mm)				
	<i>V. cholera</i>	<i>E. coli</i>	<i>P. fluorescens</i>	<i>S. typhi</i>	<i>S. aureus</i>
200	NZ	NZ	11	11	14
400	12	10	12	13	16
600	13	11	17	19	21
800	20	17	22	23	25
1000	24	22	25	26	28

5. Effect of Sodium bisulphite on Fish spoilage bacteria

The effect of Sodium bisulphite on the inhibition of growth of five fish spoilage bacteria viz., *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas fluorescens* was studied and the results were shown in Table - 5. The diameter of the inhibition zone of all the bacterial culture was increased with increase in concentrations of Sodium bisulphite from 200 to 1000 $\mu\text{g ml}^{-1}$

ml^{-1} . All the concentrations showed inhibitory zone against all the test bacterial isolates. Among the five bacterial cultures tested, Sodium bisulphite highly inhibited the growth of *Staphylococcus aureus* by showing a 36 mm zone of inhibition followed by *Salmonella typhi* (33 mm), *Pseudomonas fluorescens* (31 mm) and *Vibrio cholerae* (30 mm). The least zone of inhibition was observed in *Escherichia coli* (28 mm).

Table 5: Effect of Sodium bisulphite on the inhibition of Fish spoilage bacteria

Sodium bisulphite ($\mu\text{g ml}^{-1}$)	Diameter of inhibition zone (in mm)				
	<i>V. cholera</i>	<i>E. coli</i>	<i>P. fluorescens</i>	<i>S. typhi</i>	<i>S. aureus</i>
200	13	12	15	17	19
400	17	14	18	19	21
600	19	17	23	25	27
800	26	23	28	30	31
1000	30	27	31	33	34

6. Effect of Potassium bisulphite on Fish spoilage bacteria

The effect of Potassium bisulphite on the inhibition of growth of five fish spoilage bacteria was tested and the results were presented in Table - 6. The diameter of the inhibition zone of all the bacterial culture was increased with increase in concentrations of Potassium bisulphite from

200 to 1000 $\mu\text{g ml}^{-1}$. No zone of inhibition was observed at 200 $\mu\text{g ml}^{-1}$ against *Vibrio cholerae* and *Escherichia coli*. Potassium bisulphite exhibited maximum zone of inhibition against *Staphylococcus aureus* (27 mm) followed by *Salmonella typhi* (25 mm), *Pseudomonas fluorescens* (24 mm) and *Vibrio cholerae* (23 mm). The least zone of inhibition was observed in *Escherichia coli* (21 mm).

Table 6: Effect of Potassium bisulphite on the inhibition of Fish spoilage bacteria

Potassium bisulphite ($\mu\text{g ml}^{-1}$)	Diameter of inhibition zone (in mm)				
	<i>V. cholera</i>	<i>E. coli</i>	<i>P. fluorescens</i>	<i>S. typhi</i>	<i>S. aureus</i>
200	NZ	NZ	10	13	13
400	10	9	12	15	15
600	12	10	17	18	20
800	19	16	21	23	24
1000	23	21	24	25	27

NZ – No Zone of inhibition

Omojowo *et al.* (2009) [3] compared the salts of metabisulphite and sorbate with concentrations of 1 – 3 % on the safety and shelf life of smoked catfish. They reported that both are good in reducing the *Escherichia coli* and *Streptococcus* sp. They also found that potassium sorbate did not affect *Staphylococcus* count throughout storage while only 5 % sodium metabisulphite was able to reduce the *Staphylococcus* count to 0 and remained 0 until the end of the 8th week of storage.

Conclusion

The present study concludes that the Chemical preservatives are of predominant efficacy in the preservation of fish spoilage from the bacterial isolates. Different chemical preservatives viz., Sodium nitrite, Potassium nitrite, Sodium bisulphite, Sodium metabisulphite, Potassium bisulphite, and Potassium metabisulphite are effective in the control of fish spoilage causing bacteria like *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas fluorescens*. The Sodium metabisulphite was highly effective in the control of fish spoilage causing bacteria when compared to other chemical preservatives. Among the pathogens tested, *Staphylococcus aureus* was found to be more susceptible to chemical preservatives and *Escherichia coli* was found to be less susceptible.

References

1. Akinyemi AA, Buoro OO. Occurrence of bacteria found in gills, skin, buccal cavity of *Lutjanus agennes*, *Pseudotolithus elongatus* and *Sphyrna barracuda* from Lagos Lagoon, Nigeria, 2011;10(5):15-28.
2. Ojewola GS, Annah SI. Nutritive and economic value of danish fish meal, crayfish dust meal and shrimp waste meal inclusion in broiler diets. International Journal of Poultry Science, 2006;5(7):390-394.
3. Omojowo FS., Idris GL, Ihuahi JA. Comparative Assessment of potassium sorbate and sodium metabisulphite on the safety and shelf life of smoked catfish. Nature Science, 2009;7(8):10-17.
4. Ravichandran S, Kumaravel K, Rameshkumar G, Ajith Kumar TT. Antimicrobial peptides from the marine fishes. Research Journal of Immunology, 2010;3(8):146-156.
5. Sutharshiny S, Sivashanthini K. Total lipid and cholesterol content in the flesh of the five important commercial fishes from waters around Jaffna Peninsula, Sri Lanka. International Journal of Biological Chemistry, 2011;6(3):161-169.
6. Wefky S, Ghobrial M. Studies on the bioactivity of different solvents extracts of selected marine macroalgae against fish pathogens. Research Journal of Microbiology, 2008;3(5):673-682.