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Nisin peptide as promising natural food preservative for food

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ABSTRACT

Sodium Nitrite has been widely use as preservative for meats and fish Products, but in recent years there has been considerable interest for searching about natural Food preservatives like Nisin peptide. The aim of this study was to compare the inhibitory effect of two preservatives were Sodium Nitrite and Nisin peptide separately against Staphylococcus Aureus (Staph. A), Escherichia coli (E. coli), and Candida Albicans (C. Albicans) in Mueller Hinton Broth (MHB) at three different pH (7.0- 6.0- 5.5). After that the combination effect between Sodium Nitrite and Nisin was studied in MHB at optimum pH that was concluded from the previous stage. Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC) of both preservatives were evaluated. FIC values (Fractional Inhibitory Concentration) were calculated after combination between them. The results showed that MIC values of Sodium Nitrite against S. Aureus, E. coli and C. albicans at pH 5.5 were (500- 200- 500) ppm respectively and MIC values of Nisin were (100- 350- 500) ppm respectively, while MIC values of the combination (Sodium Nitrite+ Nisin) against S. A, E. coli and C. albicans were (50- 25- 100) ppm respectively and FIC values of them were (0.39- 0.15- 0.30). On the other hand The results showed that simultaneous use of Nisin with sodium nitrite reduced MIC and MBC of this compound against bacteria and fungi Significantly consequently, this synergistic effect of Nisin could promote in the Future to reduce of the using of Sodium Nitrite in food industry.

Key words: Nisin, Synergism, Bacteria, MIC, FIC.

INTRODUCTION

Sodium Nitrite is one of the oldest chemical food preservatives and is used commercially in food industry as coloring and flavoring agent for meat and fish products [1]. But in 1960 it was noted that the application of this compound followed by liver toxicity of some animals that was fed canned fish meals which contains high levels of Sodium Nitrite, also nitrite compounds can convert in gastro intestinal tract to give a nitrous amine (carcinogenic compound) which is responsible for Malignant tumors, besides to the Nitrates compound that can cause Met-hemoglobin phenomenon in foods, which is created by oxidation of Oxy-hemoglobin to Ferry- hemoglobin and it can be fatal especially for newborn infants [2].

Due to the previous harmful side effects of chemical preservatives like sodium Nitrite recently, it has started to search about natural food preservatives that does not have undesired side effects. Nisin Peptide is a natural food preservatives and is a member of Bacteriocin family (antibacterial peptides that were produced by bacteria can kill or inhibit the growth of other bacteria) [3]. Nisin is not a toxic and is produced during food fermentation by Lactococcus Lactis bacteria, so it can improve the smell and flavor of food products, it was approved to use as food preservative in 1969 by Joint (FAO/WHO) and given the Europe number (E 234), currently it is used in over 50 country around the world like Australia and New Zealand [4-2].

Chemically Nisin is a poly peptide composed of 34 amino acids, is effective against gram positive bacteria and spores and it has a low little efficiency against gram negative bacteria and fungi [4-5].
The Mechanism of Nisin is abbreviated by formation a complex with Lipid 2 (a precursor molecule that contribute to form bacterial cell walls), after that the Nisin - Lipid 2 complex inserts into the cytoplasmic membrane to form pores and allow the efflux of essential cellular components resulting the inhibition of the cell bacteria or death finally [4].

The objective of the present study is evaluated the potency of Sodium Nitrite and Nisin against bacteria and fungi and then study the combination effect between them to conclude if Nisin can decrease MIC and MBC values of Sodium Nitrite against bacteria and fungi or not.

EXPERIMENTAL SECTION

1- Stock solution of preservatives:
The preservatives used in the experiment were as follows:

- **Sodium nitrite** (Merck/ Germany), it was prepared by concentration 20 mg/ml (Distilled water as suitable solvent).
- **Nisin** (Aowei/ China), it was prepared by concentration 10 mg/ml (it was dissolved by HCL 2% as Suitable solvent).
- Each of solution was sterilized separately by Millipore filter with a diameter of 0.22 micrometers (Jinteng/ China).

2- Microorganism

- They were a reference strains that were obtained from University Almowassa hospital in Damascus, they were *Staph. Aureus*, *E. coli* and *C. Albicans*, it were made primary suspensions of them with Trypticase Soy Broth (Sigma/ USA) and were incubated at 37 C° for 24 hours.
- It were prepared suspensions of bacteria by concentration 5×10^5 cfu/ml (based on turbidity of the 0.5 McFarland standard) and it was prepared at concentration ($5\times^3 10$) cfu/ml for *C. Albicans*

3- MIC and MBC Tests:
This study has described the determination of MIC of both of Nisin and Sodium Nitrite against bacteria and fungi by detecting the lowest concentration of an antimicrobial agent that completely inhibits growth of the organism in the tubes as detected by the unaided eye [6], and MBC of both preservatives by detected the lowest concentration that inhibits bacterial growth rate up to 99.9% [2], and fungal growth rate up to 100%. based on macro dilution methods and according to NCCLS (National Committee for Clinical Laboratory Standards Institute) [6] as follows :

- First, it was detected MIC and MBC of Sodium Nitrite and Nisin alone Against bacteria and fungi in MHB (Biolab/ Hungary) prepared at three different pH were (7.0- 6.0- 5.5) and serial dilutions of Sodium Nitrite prepared at concentrations: (4500 - 3500- 2500- 1500- 750- 500- 350- 200- 100- 50- 25- 10) ppm, while for Nisin were (2500- 1500- 750- 500- 350- 200- 100- 50- 25- 10) ppm. The aim of this step for detection the best Effectiveness of each preservative at optimum pH and then selection later to study the combination effect between preservatives. against bacteria fungi.

- Second, serial dilutions of Sodium Nitrite were prepared in MHB at optimum pH by concentrations (350- 200- 100- 50- 25- 10) ppm, then Nisin peptide was added at MIC concentration, and MIC and MBC values were evaluated.

- The synergism of preservatives was evaluated by calculating FIC according to the formula:

$\boldsymbol{FIC = \frac{MIC1}{MIC*1} + \frac{MIC2}{MIC*2}}$ and the types of effects are classified as follows:

FIC ≤ 0.5: (synergism)
FIC = 0.5-1: (additive effect)
FIC = 1-4: (indifferent effect)
FIC> 4 : (antagonism) [7].

RESULTS AND DISCUSSION

First stage:

- The values of MIC of Sodium Nitrite and Nisin peptide against S. Aureus at pH 7.0 were (2500- 350) ppm respectively, at pH 6.0 were (750- 200) ppm respectively and at pH 5.5 were (500- 100) ppm respectively

- The values of MIC of Sodium Nitrite and Nisin peptide against E. coli at pH 7.0 were (750- 1500) ppm respectively, at pH 6.0 were (500- 750) ppm respectively, and at pH 5.5 were (200- 350) ppm respectively.

- The values of MIC of Sodium Nitrite and Nisin peptide against *Candida Albicans* at pH 7.0 were (3500- 1500) ppm respectively, at pH 6.0 were (1500- 750) ppm respectively, and at pH 5.5 were (500- 500) ppm respectively, Both table [1] and table [2], showed MIC and MBC values of Sodium Nitrite and Nisin at pH (7.0- 6.0- 5.5).

Table [1] : MIC and MBC values of Sodium Nitrite against *S. Aureus, E.coli, C. Albicans* at pH (7.0 - 6.0 - 5.5)

Microorganism	pH					
	7.0		6.0		5.5	
	MIC	MBC	MIC	MBC	MIC	MBC
Staph. Aureus	2500	3500	750	1500	500	500
E. coli	750	750	500	500	200	350
C. albicans	3500	4500	1500	2500	500	750

Table [2]: MIC and MBC values of Nisin Peptide against *S. Aureus, E. coli, C. Albicans* at pH (7.0 - 6.0 - 5.5)

Microorganism	pH					
	7.0		6.0		5.5	
	MIC	MBC	MIC	MBC	MIC	MBC
Staph. Aureus	350	750	200	500	100	350
E. coli	1500	2500	750	1500	350	500
C. albicans	1500	2500	750	1500	500	750

Second Stage:

- According to the previous results it was concluded that optimum pH was 5.5 because MIC and MBC values of both preservatives were reduced in significantly and the best effectiveness of both Sodium Nitrite and Nisin peptide obtained at this pH, so it was selected to study the combination effect between Sodium Nitrite and Nisin.

- The values of MIC and MBC of the combination (sodium Nitrite+ Nisin) against S. Aureus, E. coli and C. Albicans were (200- 100- 350) ppm respectively, while FIC values were (0.39- 0.15- 0.30). Table [3], showed MIC, MBC, FIC values of the combination (Sodium Nitrite+ Nisin) and Figure [1], showed the effect of Sodium Nitrite before its combination with Nisin and after that against *S. Aureus, E. coli* and *C. Albicans* in MHB at pH 5.5.

Table [3]:MIC, MBC, FIC values of Sodium Nitrite + Nisin in MHB at pH= 5.5

Microorganism	MIC	MBC	FIC	Effect
Staph. Aureus	50	200	0.39	Synergism
E. coli	25	100	0.15	synergism
C. albicans	100	350	0.30	synergism

The results showed that Sodium Nitrite had the best effective against gram negative bacteria *E. coli* (MIC= 200 ppm), in comparison with gram positive bacteria S. Aureus and fungi *C. Albicans* (MIC= 500 ppm) in MHB at pH= 5.5, and this Approach to the study [7], that demonstrated the potency of Sodium Nitrite against E. coli and *Pseudomonas aeruginosa*, while Sodium Nitrite had a little efficiency against *C. albicans*. Nisin was more effective against S. Aureus in MHB at pH= 5.5 (MIC= 100 ppm) than *E. coli* and *C. albicans* (MIC= 350 ppm) and these results Approach to the study[2], that demonstrated the high potency of it against *S. Aureus.*

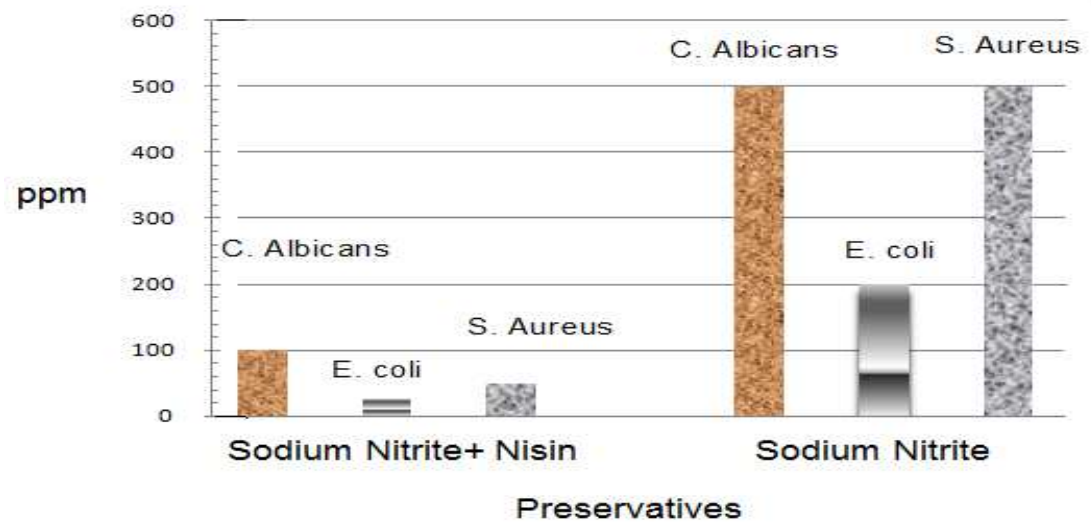

Figure [1]: The effect of Sodium Nitrite before its combination with Nisin and after that against *S. Aureus, E. coli* and *C. Albicans* in MHB at pH 5.5

MIC and MBC values of the combination (Sodium Nitrite + Nisin) was reduced clearly against S. Aureus (50 ppm), E. coli (25 ppm), C. albicans (100 ppm) bacteria and fungi, in comparison with these values that resulted after the using of each preservative alone. On the other hand, Nisin peptide could reduce MIC and MBC values of Sodium Nitrite against S. Aureus, E. coli and C. albicans in percentage by 86%- 75%- 80% respectively also, all FIC values for S. Aureus, E. coli and C. albicans were (0.39-0.15-0.30) respectively < 0.5, which it means that there were synergism effect between Sodium Nitrite and Nisin peptide, and that Approach to study (Hamed Haddad Kashani etal: 2012), that demonstrated on synergism effect against S. Aureus and Listeria Monocytogenes.

CONCLUSION

The synergism between Sodium Nitrite and Nisin could encourage to use it in the food industry and decrease of the amounts of Sodium Nitrite and its harmful effects in food in the future.

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