Effect of Some Chemical Preservatives on Harmful Bacteria in Meat products

Saad, M. Saad[1]; Faten, S.Hassanin[1]; Amani, M.Salem[1], Nahla, A.Shawqy[2] and Walaa, M.Gomaa[2]

[1] *Food Control Department. Faculty of Veterinary Medicine, Benha University*

[2] *Animal Health Research Institute, Shebin el koom Branch, Egypt.*

A B S T R A C T

Food preservation is designed to enhance or protect food safety while maintaining or improving product quality by inactivating or inhibiting the growth of undesirable microorganisms. A total of 6600 g fresh minced beef samples were divided into 11 equal groups (600 g of each); each group was subdivided into 3 samples (each one 200 gm) for counting *S.aureus. S.aureus* was inoculated into each group with infective dose 10^6 cfu /g.The used chemical preservatives were sodium nitrite (50 ppm - 100 ppm), nisin (40ppm - 60ppm) and potassium sorbate (0.2% – 0.3%) singly and in combinations in different concentrations were added and the inoculated samples were kept at 4 °C and examined every 3-6-9-12-24-48h for *S.aureus* count. The experiment was performed in triplicate. Generally, A combination of sodium nitrite (100 ppm) with nisin (60 ppm) and potassium sorbate (0.3%) proved to be more efficient than others where it reduced *S. aureus* count (cfu/ g) from $5.2 \times 10^6 \pm 4.7 \times 10^4$after 3h to $5.8 \times 10^2 \pm 1.0 \times 10^2$after 48h. Therefore**,** the use of this combination is recommended to improve the safety of meat products.

Keywords: *S.aureus*, meat products, nisin, potassium sorbate

(http://www.bvmj.bu.edu.eg) **(BVMJ-34(1): 353-359, 2018)**

1. INTRODUCTION

Meat is a nutritious protein-rich food which is highly perishable and has a short shelf-life unless preservation methods are used. However, it gets easily contaminated by pathogenic microorganisms present in animal prior to slaughter.

Staphylococcus aureus is a leading cause of food poisoning resulting from the consumption of contaminated food with staphylococcal enterotoxins. Different foods can act as a good medium for *Staph. aureus* such meat products (Guven et al., 2010). Presence of *Staph. aureus* in meat products may be attributed to direct contact with workers with hand or arm lesions caused by *Staph. aureus*, or by coughing and sneezing, which is common during respiratory infections. Food handlers are frequently the source of food contamination in staphylococcal outbreaks (Jennifer Hait, 2012).

Food preservation is designed to enhance or protect food with maintaining or improving the product quality. Through inactivating or inhibiting the growth of undesirable microorganisms. Many methods of preservation are available to food processors including thermal processing, refrigeration, addition of chemical preservatives or a combination of several of these methods (Sabreen and Enas, 2001).

Whereas, nisin is an antimicrobial peptide produced by some strains of *Lactococcus lactis* and used in meat technology as a chemical preservative where it has a powerful inhibitory effect against Gram positive bacteria, but probably has not the same effect on Gram-negative bacteria as *E.coli* (Delves & Gasson, 1994 and Thomas et al., 1998) and nitrite and nitrate in meat products provide three desirable properties to meat to which it is added. First, it stabilized the red to pink coloration commonly associated with nitrite cured meat. Secondary nitrite enhances meat product flavor through retardation of degradation which inhibited by oxidation. The third function is inhibiting toxin production by *Clostridium botulinum* (Gray *et al*, 1981). And also sorbate increased the lag phase and decreased the growth rate of bacteria during the exponential phase and significantly lengthened the time of aerobic counts on vacuum-packaged beef to reach 10^6 cfu/cm2 (Zamora and Zaritzky, 1987).

Therefore, the present study was applied to investigate the effect of nisin (40- 60 ppm), sodium nitrite (50 and 100 ppm) and potassium sorbate (0.2% and 0.3%) on *S.aureus* artificially inoculated into minced meat samples.

2. Materials and methods

2.1. Strain used:

S. aureus strain which obtained from Animal Health Research Institute, Dokki, Giza, governorate with the recommended infective dose 10^6 cfu/g.

2.2. Preservatives used as recommended *by Hassan (1999):*

Nisin at concentrations of (40 and 60 ppm)

Sodium nitrite (50 and 100 ppm)

Potassium sorbate (0.2% and 0.3%)

2.3. Experimental application:

Accurately, 6600 g of fresh minced meat samples were divided into 11 equal groups (600 g of each), Every group was subdivided into 3 samples (each weighing 200 g) for counting *S.aureus.* Accordingly, *S.aureus* was inoculated into each group with infective dose 10^6 cfu /g. The used chemical preservatives were added according to the following order:

1st: control (+ve) inoculated by *S.aureus* strain only without any preservatives

*2^nd^*: *S.aureus* strain + 0.8 ml nisin(40 ppm).

3rd: *S.aureus* strain + 1.2 ml nisin(60 ppm).

4th: *S.aureus* strain + 2 ml sodium nitrite (50 ppm).

5th: *S.aureus* strain + 2.5 ml sodium nitrite (100 ppm).

6th: *S.aureus* strain + 0.4g potassium sorbate (0.2%).

7th: *S.aureus* strain + 0.6g potassium sorbate (0.3%).

8th: *S.aureus* strain + 2 ml sodium nitrite + 0.8 ml nisin.

9th: *S.aureus* strain + 2 ml sodium nitrite + 0.4 ml potassium sorbate.

10th: *S.aureus* +2 ml sodium nitrite + 0.8 ml nisin + 0.4 ml potassium sorbate.

11th: *S.aureus* strain + 2.5 ml sodium nitrite + 1.2 ml nisin + 0.6 ml potassium sorbate.

The samples after inoculation were kept at 4 °C till be used. The inoculated groups were examined every 3-6-9-12-24-48h for *S.aureus* count. The experiment was performed in triplicate.

2.4. Statistical analysis:

The statistical analysis of this study was carried out according to *Snedecor and Cochran (1967).*

3. RESULTS

Tables (1 & 2) illustrated the effects and reduction percentages of nisin (40- 60ppm), Sodium nitrite (50 and 100 ppm) and Potassium sorbate (0.2% and 0.3%) on *S.aureus* artificially inoculated into minced meat samples. Also, Nisin (40- 60ppm) decreased the *Staph aureus* counts from 5.0 x 10^6 (initial load) to 3.4 x 10^6 ± 2.1 x 10^5, 2.5 x 10^6 ± 1.2 x 10^5, 2.3 x 10^6 ± 4.3 x 10^5, 1.0 x 10^6 ±5.8 x 10^5, 7.0 x 10^5 ± 7.0 x 10^4 and 6.1 x 10^5 ± 5.0 x 10^4 cfu/g with reduction percentages 37.04%, 51.92%, 51.92%, 81.48%, 86.54% and 88.04% after 12h, 24h and 48h of storage, respectively. Moreover, Sodium nitrite (50- 100 ppm) decreased the *Staph aureus* counts from 5.0 x 10^6 (initial load) to 1.8 x 10^6 ± 6.3 x 10^5, 1.6 x 10^6 ± 1.4 x 10^5, 1.3 x 10^6 ± 2.0 x 10^5, 2.4 x 10^6 ± 1.2 x 10^5, 1.2 x 10^6± 8.3 x 10^5 and1.0 x 10^6 ± 3.4 x 10^5CFU/g with reduction percentages 66.67%, 69.23%, 74.51%, 55.55%, 76.92% and 80.39% after 12h, 24h and 48h of storage, respectively. And also, Potassium sorbate (0.2-0.3 %) decreased or increased the *S. aureus* counts from 5.0 x 10^6 (initial load) to 4.1 x 10^6 ± 5.7 x 10^5, 4.8 x 10^6 ± 4.0 x 10^5, 7.3x 10^6 ± 6.5 x 10^5, 6.0 x 10^6 ± 4.5 x 10^5, 3.2 x 10^6 ± 4.5 x 10^5 and 8.2 x 10^6 ± 4.5 x 10^6cfu/g after 12h, 24h and 48h of storage, respectively. with increasing percentage 43.14% and 60.78% after 48h of storage, respectively.

The combination of 40 ppm nisin and 50ppm sodium nitrite decreased the *S. aureus* counts from 5.0 x 10^6 (initial load) to 3.4 x 10^5 ± 4.4 x 10^4, 2.0 x 10^5 ± 1.4 x 10^5 and 3.7 x 10^4 ± 2.7 x 10^3CFU/g with reduction percentages 93.70%, 96.15% and 99.27% after 12h, 24h and 48h of storage, respectively. While Combination of 50 ppm sodium nitrite and 0.2% potassium sorbate decreased the *S. aureus* counts from 5.0 x 10^6 (initial load) to2.6 x 10^6 ± 6.7 x 10^5, 1.7 x 10^6 ± 6.0 x 10^5 and 4.4 x 10^5 ± 1.0 x 10^5CFU/g with reduction percentages 51.85%, 67.31% and 91.37% after 12h, 24h and 48h of storage, respectively. And also, combination of sodium nitrite (50 ppm) + nisin (40 ppm) + potassium sorbate (0.2%) decreased the *S. aureus* counts from 5.0 x 10^6 (initial load) to 6.5 x 10^5 ± 1.0x 10^5, 4.2x 10^4 ± 1.3 x 10^4 and 3.2 x 10^4 ± 1.4 x 10^4 cfu/g with reduction percentages 87.96%, 99.19% and 99.37% after 12h, 24h and 48h of storage, respectively. Moreover, combination of sodium nitrite (100 ppm) + nisin (60 ppm) + potassium sorbate (0.3%) decrease the *S. aureus* counts from 5.0 x 10^6 (initial load) to 4.2 x 10^4 ± 1.5 x 10^4, 1.0 x 10^4 ± 6.8 x 10^3 and 5.8 x 10^2 ± 1.0 x 10^2 cfu/g with reduction percentages 99.22%, 99.81% and 99.99% after 12h, 24h and 48h of storage, respectively. While in control samples, *S.aureus* count increased from 5.0 x 10^6 (initial load) to 5.1 x 10^6

Table (1): The effect of various concentrations of nisin, sodium nitrite and potassium sorbate on the counts of *Staph. aureus* (CFU/g) artificially inoculated into minced meat samples

Groups	After 3h	After 6 h	After 9 h	After 12 h	After 24 h	After 48 h
Control +ve	5.3 x 106	5.4 x 106	5.4 x 106	5.4 x 106	5.2 x 106	5.1 x 106
nisin (40 ppm).	5.1 x 106 ±1.6 x 105	6.1 x 106 ± 3.2 x 105	5.6 x 106 ± 2.2 x 105	3.4 x 106 ± 2.1 x 105	2.5 x 106 ± 1.2 x 105	2.3 x 106 ± 4.3 x 105
nisin (60 ppm).	4.7 x 106 ± 2.4 x 105	4.6 x 106 ± 4.1 x 105	1.7 x 106 ± 7.0 x 105	1.0 x 106 ±5.8 x 105	7.0 x 105 ± 7.0 x 104	6.1 x 105 ± 5.0 x 104
sodium nitrite (50 ppm).	2.7 x 106 ± 6.5 x 105	3.8 x 106 ±5.2 x 105	3.1 x 106 ± 1.0 x 105	1.8 x 106 ± 6.3 x 105	1.6 x 106 ± 1.4 x 105	1.3 x 106 ± 2.0 x 105

sodium nitrite (100 ppm).	5.1 x 106 ± 2.5 x 105	7.0 x 106 ± 3.5 x 105	5.8 x 106 ± 1.0 x 107	2.4 x 106 ± 1.2 x 105	1.2 x 106± 8.3 x 105	1.0 x 106 ± 3.4 x 105
potassium sorbate (0.2%).	5.0 x 106 ± 5.6 x 105	3.7 x 106 ± 2.0 x 105	4.1 x 106 ± 1.0 x 105	4.1 x 106 ± 5.7 x 105	4.8 x 106 ± 4.0 x 105	7.3x 106 ± 6.5 x 105
potassium sorbate (0.3%).	3.6 x 106 ± 6.7 x 105	4.8 x 106 ± 1.1 x 105	4.1 x 106 ± 1.4 x 105	6.0 x 106 ± 4.5 x 105	3.2 x 106 ± 4.5 x 105	8.2 x 106 ± 4.5 x 106
sodium nitrite (50 ppm) + nisin (40 ppm).	5.0 x 106 ± 1.7 x 105	4.2 x 106 ± 1.8 x 105	2.2 x 106 ± 6.1 x 105	3.4 x 105 ± 4.4 x 104	2.0 x 105 ± 1.4 x 105	3.7 x 104± 2.7 x 103
sodium nitrite (50 ppm) + potassium sorbate (0.2%).	5.2 x 106 ± 4.6 x 104	4.3 x 106 ± 2.3 x 105	2.6 x 106 ± 1.3 x 106	2.6 x 106 ± 6.7 x 105	1.7 x 106 ± 6.0 x 105	4.4 x 105± 1.0 x 105
sodium nitrite (50 ppm) + nisin (40 ppm) + potassium sorbate (0.2%).	5.2 x 106 ± 7.7 x 104	3.8 x 106 ± 4.6 x 105	4.2 x 106 ± 4.8 x 105	6.5 x 105 ± 1.0x 105	4.2x 104 ± 1.3 x 104	3.2 x 104 ± 1.4 x 104
sodium nitrite (100 ppm) + nisin (60 ppm) + potassium sorbate (0.3%).	5.2 x 106 ± 4.7 x 104	3.5 x 106 ± 8.0 x 105	5.4 x 105± 8.1 x 104	4.2 x 104 ± 1.5 x 104	1.0 x 104 ± 6.8 x 103	5.8 x 102± 1.0 x 102

Intial load of S.aures = 5.00 x 106 cfu/g. The values represent mean ± SD of three experiments.

Table (2): Reduction % of *Staph. aureus* (CFU/g) artificially inoculated into minced meat samples treated with different concentrations of nisin, sodium nitrite and potassium sorbate

Groups	After 3h	After 6 h	After 9 h	After 12 h	After 24 h	After 48 h
nisin (40 ppm)	3.77	12.96 (I R)	3.70 (I R)	37.04	51.92	54.91
nisin (60 ppm)	11.32	14.81	68.52	81.48	86.54	88.04
sodium nitrite (50 ppm)	49.05	29.63	42.59	66.67	69.23	74.51
sodium nitrite (10 ppm)	3.77	29.63 (I R)	7.41 (I R)	55.55	76.92	80.39
Potassium sorbate (0.2%)	5.66	31.48	24.07	24.07	7.69	43.14 (I R)
potassium sorbate (0.3%)	32.07	11.12	24.07	11.11 (I R)	38.46	60.78 (I R)
sodium nitrite (50 ppm) + nisin (40 ppm).	5.66	22.22	59.26	93.70	96.15	99.27

sodium nitrite (50 ppm) + potassium sorbate (0.2%).	1.89	20.37	51.85	51.85	67.31	91.37
sodium nitrite (50 ppm) + nisin (40 ppm) + potassium sorbate (0.2%).	1.89	29.63	22.22	87.96	99.19	99.37
sodium nitrite (100 ppm) + nisin (60 ppm) + potassium sorbate (0.3%).	1.89	35.18	90	99.22	99.81	99.99

I R: increasing rate

4. DISCUSSION

Meat products are perishable foods and unless stored under proper conditions spoil quickly. In addition, if pathogens are present, meat products become hazardous for consumers. Therefore, assurance of meat safety and quality is the most important (Shimoni and Iabuza, 2000).

Food preservation is designed to enhance or protect food safety while maintaining or improving product quality by inactivating or inhibiting the growth of undesirable microorganisms (Ray, 1992).

Food preservation techniques can cause a variety of stresses that interfere with bacterial homeostasis to prevent growth or to kill bacteria. However, as a result of the stress response, some bacteria can survive and grow after the application of stress (Jones and Inouye, 1994).

The antioxidant effect of nitrite is likely due to the same mechanisms responsible for cured color development involving reactions with heme proteins and metal ions, chelating of free radicals by nitric oxide, and the formation of nitriso- and nitrosyl compounds having antioxidant properties (Sebranek, 2009).

Sorbic acid and its salts have several advantages as food preservatives. Initially thought to have only antimycotic activity, but now they known that they are able to inhibit a wide range of bacteria, particularly aerobic catalase-positive organisms. Effective concentrations do not normally alter product taste or odor. These preservatives are also considered harmless. Potassium salt is commonly used because it is more stable. Furthermore, its greater solubility extends the use of sorbate to solutions appropriate for dipping and spraying (Thomas, 2000).

In this study, there is decrease in the viable count of *S. aureus* especially after 24 and 48 hours.

Abdel-Shakour *et al*. (2014) reported that when nisin was added as food preservative at 30 ppm, no growth was observed for Gm +ve bacteria as *S. aureus* and also reported that *S. aureus as* Gm+ve bacteria showed high sensitivity to sodium nitrite higher than other Gm-ve strains.

It is evident from the results that addition of potassium sorbate in concentrations (0.2%, 0.3%) had no effect on the viable count of inoculated *S. aureus* in minced meat samples during the experimental period (48 h)

Using several preservatives combinations, results in more inhibitory effect on microbiological growth as that) and reported by Abdel-Shakour *et al.* (2014) who declared that when sodium nitrite 125 ppm was added in combined with nisin 10 ppm there was an inhibitory effect against *S. aureus* as no growth observed, whereas used combination of three preservatives revealed greater decline the count of inoculated *S. aureus* with increasing concentration of preservatives (adding of 50ppm sodium nitrite with 40ppm nisin and 0.2% potassium sorbate).

While results of using combination of 100 ppm sodium nitrite with 60 ppm nisin and 0.3% potassium sorbate revealed the greatest inhibition of inoculated *S. aureus*

5. CONCLUSION

Preservatives in certain concentrations and exact combinations of several preservatives have a great role in inhibiting and reduction microbial growth that have public health hazards.

Therefore, using a combination of sodium nitrite (100 ppm) with nisin (60 ppm) and potassium sorbate (0.3%) proved to be more efficient on *S.aureus* growth than others. So, it was significantly than using of each preservative alone in controlling the growth of food borne bacteria and improving the quality and safety of meat products.

5- REFERENCES

Abdel-Shakour, E. H.; Elouboudy, S. S.; Abdelaziz, Z. K.; Hassan, M. A.; and Emara, M.B. 2014. “Application of Bacteriocin as Bio-preservative in Foods” *Adv. J. N Y Sci* ., 7(6):87-93.

Delves, B. and Gasson, M. 1994. Nisin. In:Natural antimicrobial systems in food Preservation (eds. V.M.Diallon and R.G.Board). CAB International, Wallingford, United Kingdom.

E.O.S.Q.C (Egyptian Organization for Standarization and Quality Control) (2005): Egyptian standards for requirements of sausage, No: 1972. Egyptian standards for requirements of beef burger, No: 1688. Egyptian standards for requirements of minced meat, No: 1694. Egyptian standards for requirements of luncheon meat, No: 1114

Gray,J.L; McDonald,B.; Pearson,A.M. and MortanJ.D, 1981. "Role of nitrite in cured meat flavour : A review". J. Food Prot., 44: 302.

Guven, K.; Mutlu, B.M.; Gulbandilar, A. and Cakir, P. 2010. Occurrence and characterization of *S. aureus* isolated from meat and dairy products consumed in Turkey. J. Food Safety, 30: 196-212.

Hassan, M.A. 1999. Follow up of some pathogens in meat products and their resistance to certain preservatives. Beni-Suef Vet.Med.J., 9 (3): 417-429.

Jennifer Hait, B. S. 2012. Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins. *Staphylococcus aureus*. In Bad Bug Book, (ed.2).

Jones, P.G. and Inouye, M. 1994. “The cold-shock response- a hot topic. Molec. Microbiol. J., 11: 811-818.

Ray, B. 1992. "Nisin of *Lactococcus lactis sub sp. Lactis* as a food bio-preservative", pp 207-264. In: Food Biopreservatives of microbial origin. CRC Press, Boca Raton, Fla.

Sabreen,M,S. and Enas,E.P. 2001."Prevalence of *Escherichia coli* and effect of different concentrations of nisin on its viability in cream".Assiut Vet.Med.J, 46 (91).

Sebranek, J. G. 2009. "Basic curing agents"., Pages 1-24 in Ingredients in Meat Products. R. Tarte, ed. Springer Science+ Business Media LLC, New York, NY.

Shimoni, E. and Labuza, T. P. 2000. Modeling pathogen growth in meat products: future challenges.Trends in food Science and Technology 11(11): 394.

Snedecor,G. W. and Cochran, W.G. 1967."Statistical Methods". 6th Ed. Oxford Publishing Company, London.

Thomas, L., Davies, E., Delves, J. and Wimpenny, J. 1998. Synergistic effect of sucrose and fatty acid esters on nisin inhibition of Gram positive bacteria. J. Appl.Microbial., 85 (6): 1013-1022.

Thomas, L. V. 2000. "Preservatives, Sorbic acid" In R. K. Robinson, C. A. Batt, & C. Patel (Eds.), Encyclopedia of food microbiology (pp. 1769-1776). New York: Academic Press.

Zamora, M. C. and Zaritzky, N. E. 1987.Potassium sorbate inhibition of microorganisms growing on refrigerated packaged beef. J. Food Sci., 52:257.

.