Combined effects of nisin and modified atmosphere packaging on chemical, microbial and sensory properties of emulsion-type sausage

Khajehali E, Shekarforoush S.S., Nazer A. H. K., Hoseinzadeh S.

Department of Food Hygiene and Public health, School of Veterinary Medicine, Shiraz University, Shiraz, 71345-1731, Iran

Abstract— **Use of nisin, as one of the most common natural antimicrobial compound, and modified atmosphere packaging (MAP) are two complementary approaches to extend the shelf-life of meat products and preserve the safety and organoleptic characteristics. In the present study, effect of nisin (30 ppm) on chemical, microbial and sensory properties of emulsion type sausage stored under two different atmospheres using the fallowing gas mixtures (%CO_2/ %N_2/ CO% /%O_2): 39.5/60/ 0.5 and 39.5/20/0.5/40 were investigated. The samples stored for 42 days at 3±1 ºC. Microbial count, values of pH, CIE L*, a* and b* color parameter, lipid oxidation and sensory properties were assessed throughout the storage period. The data showed that nisin was able to reduce the growth of mesophilic, psychrotrophic and lactic acid bacteria. However, MAP did not show any significant effects on microbial growth. MAP could produce a stabilized red color and minimize the oxidative reactions in the samples. In general, packaging conditions had no significant effect on pH and sensory properties. Therefore, the use of MAP in combination with nisin can lead to better quality preservation of the emulsion type sausages and in extending their shelf life.**

Keywords— **Modified atmosphere packaging, Nisin, Emulsion type sausage, Shelf life.**

I. INTRODUCTION

Nisin is active against gram-positive organisms including lactic acid bacteria (LAB), which are the major bacterial group responsible for spoilage of refrigerated vacuum or modified atmosphere packed cooked meat products [1]. Modified atmosphere packaging (MAP) has been applied successfully for extending shelf life and preserving quality of meat products [2]. In the technique, CO_2, O_2 and N_2 are the three main gases, which are used in different concentrations.

Therefore, bacteriocins and MAP are two comple-mentary hurdles of advantage to microbial contamin-ation of food. Using nisin as an anti-microbial agent in emulsion type sausage in combi-nation with MAP has not been compre-hensively studied, yet. Thus, the aim of our study was to investigate the effect of various gas mixtures in combination with nisin on the chemical, microbial and sensory properties of emul-sion type sausages.

II. MATERIALS AND METHOD

A. Preparation and packaging of sausages

An emulsion-type sausage was produced as described by Sham Sham meat processing industry, Shiraz, Iran. The amount of 30 ppm of nisin (Sigma, N5764).) was added to the experimental groups.

The emulsions were then stuffed in cellulose casing of 70 mm diameter, using automatic filler and then cooked in steam bath at 85 ºC for 90 minutes. After being showered with cold water, they were stored at 4 ºC for 24 h and finally pilled and sliced in 1.7 mm thickness using automatic slicer. The amount of 150 g of the sliced sausage was packed on polyethylene and polyamide trays by packaging machine (ILPRA Model food pack speedy v/g, Italy), which were subsequently filled with 500 ml of two different gas mixtures using Miscelatore machine (model IV1, Cryotek ENG CO., Italy). The package was sealed with 60 µm polyethylene and polyamide sheet (water vapour permeability 5–7 g/m^2/24 h at 23 ºC and oxygen permeability 40–50 ml/ m^2/24 h at 23 ºC).

The experimental groups were prepared as follows: G1: MAP (39.5% CO_2, 60% N_2, 0.5% CO); G2: Nisin (30 ppm) + MAP (39.5% CO_2, 60% N_2, 0.5% CO); G3: MAP (39.5% CO_2, 20% N_2, 0.5% CO, 40% O_2); G4: Nisin (30 ppm) + MAP (39.5% CO_2, 20% N_2, 0.5% CO, 40% O_2).

The samples were stored at 3 ± 1 ºC for 42 days. One sample from each experiment group was then used every 7 days of storage for microbial, chemical and sensory analysis.

B. Analysis

1. Microbiological analysis

Enumerations of psychrotrophic and mesophilic aerobic bacteria were determined by pouring the specimens on plate count agar. Man Rogosa Sharpe Medium agar and violet red bile glucose agar were used for the enumeration of LAB and enterobacteriaceae, respectively. Fungi were enumerated by surface plating on Subaru dextrose agar containing 150 mg/l tetracycline. Pseudomonads were counted by surface plating on cetrimide fusidin cephaloridine agar supplemented with SR 102 and *Brochothrix thermosphacta* was determined by surface plating on streptomycin thallous acetate cycloheximide agar.

2. pH measurement

pH measurement was performed on the diluted homogenates using micro pH meter.

3. Lipid oxidation

Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) method described by [3].

4. Color evaluation

Surface color (L*, a*, b*) of the samples was measured objectively using the Hunter L*, a*, b* system with a simple digital imaging method described by [4], 30 min after opening the package.

5. Sensory evaluation

The sausage samples were evaluated by a six-member trained panelist, as described by Djenane et al. (2001). The attributes studied were: color, odor, taste and texture [5].

II. RESULTS AND DISCUSSION

1. Microbiological analysis

Aerobic mesophile counts (AMC) during the storage time were changed from 1.76-2.86 log cfu/g to 4.24-6.07 log cfu/g. AMC was significantly influenced by the nisin ($P<0.05$) while, no significant effect was appeared using different MAP (Fig 1). This could be explained as using CO_2 can inhibit growing gram-negative bacteria [6] and also could permit the growth of gram- positive bacteria [7].

The results showed that the psychrotrophic aerobic growth in the treated groups was significantly suppressed ($P <0.05$) (Fig. 1B). Whilst, no significant effect was shown on the psychrotrophic aerobic count using different MAPs. Such changes in the microbial count might be due to the presence of LAB, which can be effectively inhibited by nisin. Samples stored without O_2 showed a lower count, which may be due to either the absence of O_2 or the presence of CO_2.

The load of LAB in groups G1 and G3 increased from 1.05-2.09 log cfu/g to about 7.7-7.13 log cfu/g during the storage time, while, they were dramatically suppressed by nisin in groups G2 and G4 ($P<0.05$) (Fig. 1C). Therefore, the MAP conditions did not influence the growth of LAB. Our results showed that, growing of gram-positive bacteria such as LAB was inhibited by nisin and thus may extend the shelf life of emulsion type sausages.

The number of yeasts and moulds were increased to 5.17-7.23 log cfu/g after 42 days of storage. The samples packaged without oxygen, had slower growth than samples packed with oxygen in both nisin treated and nisin free samples. Our results revealed that in the group of sausages exposed to gas mixture (39.5/60/0.5% CO_2 /N_2 /CO), the number of yeasts and molds were lower. The packaging in anoxic environment and the CO_2 solubility could explain these observations. Furthermore, our results demonstrated that the ineffectiveness of the nisin on growth of yeast or mold population is more likely due to the structure of their outer membrane, which does not allow molecules like nisin to reach their site of action.

The number of *Enterobacteriaceae,* Brochothrix *thermosphacta* and *Pseudomonas sp*. were remained under the detection limit for all treatment groups.

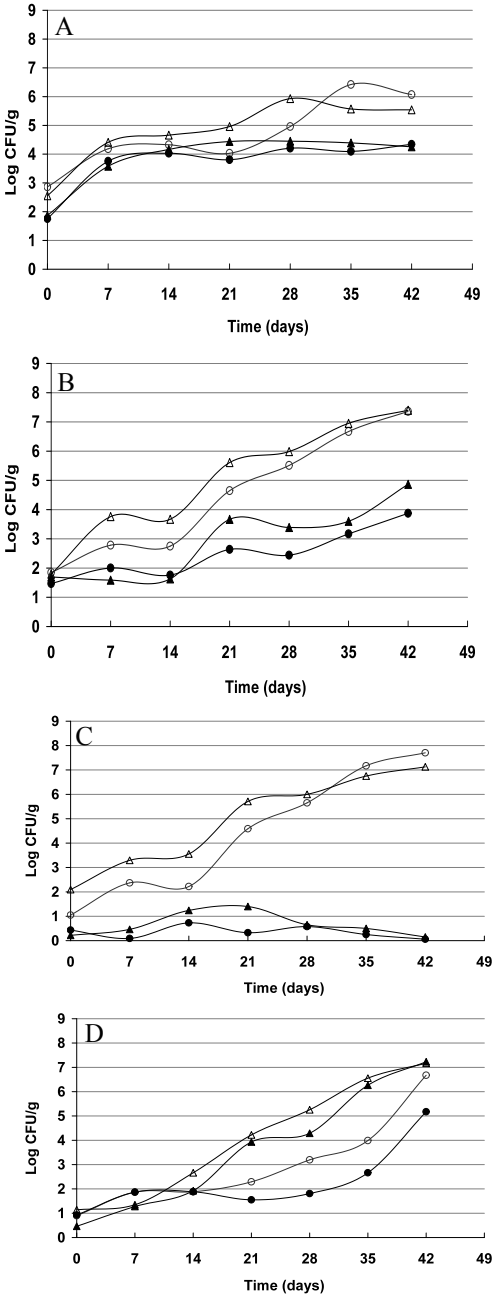

Fig1. Mesophilic (A), Psychrophilic (B), Lactic acid bacteria (C) and Fungi (D) counts (Log CFU g^{-1}) in emulsion type sausage affected by nisin and modified atmosphere packaging during 42 days of storage at 3 ± 1 ºC.

-○- MAP (39.5% CO_2, 60% N_2, 0.5% CO); -●- MAP (39.5% CO_2, 60% N_2, 0.5% CO) + 30 ppm Nisin; -Δ- MAP (39.5% CO_2, 20% N_2, 0.5% CO, 40%O_2); -▲-MAP (39.5% CO_2, 20% N_2, 0.5% CO, 40%O_2) + 30 ppm Nisin.

2. pH analysis

The initial pH of all experimental groups was ranged between 6.49 and 6.51, which remained constant during the storage period. They were affected neither by nisin nor the MAP conditions ($P > 0.05$). It was previously explained that lactic acid concentration would not start to rise until the LAB population reach about 10^7 CFU/g [8].

3. Lipid oxidation

TBARs values in all groups were significantly reduced during the period of storage which decreased from 55.67- 60.98 to 25.07- 35.20 ($P < 0.05$) (Fig. 2). The presence of some antioxidant component which was added to the vegetable oil, nitrite and CO were possibly responsible for preventing the increase in TBARs values. An antioxidant activity of CO was corroborated by Besser and Kramer (1972) who demonstrated that CO is an enzyme inactivator [9]. Fiore et al. (2007) indicated that the type of lean meat used in the formulations is an important factor for lipid oxidation due to high iron content [10].

4. Instrumental measurements of color

The samples under modified atmospheres kept L* parameter (lightness) constant during the whole period of storage. No significant differences in a* parameter (redness) were detected either during the storage periods or during the four packaging conditions.

The Storage time and packaging conditions have not affected the parameter b* (yellowness), a color parameter related to the intensity of the oxidation process. This could be due to the existence of nitrite which reacts with muscle pigment myoglobin to produce the desirable red pigment, nitrosomyoglobin, which can not act as a catalyst of lipid oxidation.

5. Sensory evaluation

Altogether, the sensorial characteristics (colour, odour, taste and texture) were reduced during the storage time ($P < 0.05$), even though, no significant differences were shown among the experimental groups.

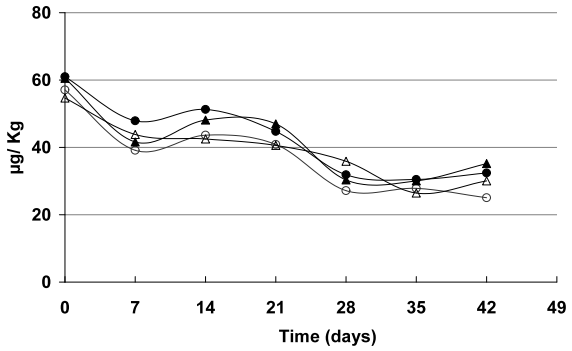

Fig. 2 TBARs (µg malonaldehyde kg^{-1}) in emulsion type sausage affected by nisin and modified atmosphere packaging during 42 days of storage at 3 ± 1 ºC.

-○- MAP (39.5% CO_2, 60% N_2, 0.5% CO); -●- MAP (39.5% CO_2, 60% N_2, 0.5% CO) + 30 ppm Nisin; -Δ- MAP (39.5% CO_2, 20% N_2, 0.5% CO, 40%O_2); -▲-MAP (39.5% CO_2, 20% N_2, 0.5% CO, 40%O_2) + 30 ppm Nisin.

IV. CONCLUSION

The results suggest that nisin can reduce the aerobic plate count and LAB in modified packaged sausages stored at low temperature. The count of yeast and mould was reduced by removing oxygen in modified atmosphere packaging. The inclusion of CO in modified atmosphere packaging delayed metmyoglobin formation, stabilized red color and minimized the oxidative reactions. In addition, packaging conditions used in this experiment did not lead to differences in pH and sensory properties. Therefore, it can be concluded the use of MAP in combination with nisin treatments, resulted in an extension of shelf life of emulsion type sausages.

ACKNOWLEDGEMENTS

This research was financially supported by "Natural antimicrobials centre of excellence". The work was also supported by Sham Sham meat processing industry.

REFERENCES

1. Samelis J, Georgiadou KG (2000) The microbial association of Greek taverna sausage stored at 4 ºC and 10 ºC in air, vacuum or 100% carbon dioxide, and its spoilage potential. J Appl Microbiol 88: 58–68.
2. Gokoglu N, Yerlikaya P, Uran H et al (2010) The effect of modified atmosphere packaging on the quality and shelf life of frankfurter type-sausages. J Food Quality 33: 367-380
3. Bostsoglou NA, Fletouris D, Papageorgiou GE et al (1994) Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. J Agri Food Chem 42: 1931–1937.
4. Yam KL, Papadakis SE (2004)A simple digital imaging method for measuring and analyzing color of food surfaces. J Food Eng 61:137–142.
5. Djenane D, Sanchez-Escalante A, Beltran JA et al (2001) Extension of the retail display life of fresh beef packaged in modified atmosphere by varying lighting conditions. J Food Sci 66: 181–186
6. Church N (1994) Developments in modified atmosphere packaging and related technologies. Trends Food Sci Technol 5: 345–352
7. Koutsoumanis KP, Geornaras I, Sofos, JN (2006) Microbiology of land muscle foods. In Hand book of Food Science, Technology and Engineering, vol. 1, (Hui Y.H ed) CRC Press, Australia
8. Korkeala H, Alanko T, Makela P et al (1990) Lactic acid and pH as quick indicators of spoilage in vacuum-packed cooked ring sausages. Int J Food Microbiol 10: 245–254
9. Besser T, Kramer A (1972) Changes in quality and nutritional composition of foods preserved by gas exchange. J Food Sci 37: 820–824
10. Fiore AG, De Pilli T, Severini C (2007) Effect of the reduction or total replacement of pork back fat with extra-virgin olive oil on quality of turkey ‘‘salami”. Proceedings of the international symposium of meat safety, From abattoir to consumer. Valencia, Spain, pp 259–264