American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 2 (3): 190-195, 2007
ISSN 1557-4571

Effectiveness of Commercial Organic Acids' Mixture (Acetolac™) to Extend the Shelf Life and Enhance the Microbiological Quality of Merguez Sausages

[1]Bouchra El Ayachi, [2]Ahmed Daoudi and [3]Noreddine Benkerroum
[1]Société Anonyme des Palmerais Koutoubia SAPAK Mohammedia, Morocco
[2]Institut Agronomique et Vétérinaire Hassan II, Département HIDAOA, BP 6202, Instituts, 10101, Rabat, Morocco
[3]Institut Agronomique et Vétérinaire Hassan II, Département des Sciences Alimentaires et Nutritionnelles, BP 6202, Instituts, 10101, Rabat, Morocco

Abstract: The effect of the addition of organic acids on the shelf life and on the hygienic quality of Merguez sausages was studied. A commercial organic acid; Acetolac™ consisting of a mixture of sodium lactate (90%) and sodium acetate (10%) was added at different concentrations (0, 5, 10, 15 or 20 g kg^{-1}) to the butter mixture for sausage making. Microbiological enumerations (total aerobic count, coliforms, fecal streptococci, *Staphylococcus aureus* and sulfite reducing clostridia) along with the pH and Total Volatile Basic nitrogen (TBV-N) were monitored during storage at refrigeration temperature (~8°C) for 15 days. The results showed that the addition of organic acids reduced significantly ($p<0.05$) the microbiological counts during the first three days of storage and such an effect increased with the concentration of added Acetolac™. Thereafter, a significant ($p<0.05$) increase in the colony forming units (cfus) of all the bacterial groups enumerated was recorded with the exception of *S. aureus* whose cfus remained practically constant. Concomitantly, the pH and TBV-N content have increased throughout the whole period of storage. As for the shelf life, control samples (without added Acetolac™) were altered at day 5 of cold storage as judged by a sensory panel on the basis of perceivable sensory attributes. However, such perceivable alterations were delayed at least 5 days in samples treated Acetolac™ at levels ranging between 10 and 20 g kg^{-1}.

Key words: Acetolac™, coliforms, fecal streptococci, *Staphylococcus aureus*, sulfite reducing clostridia, merguez, shelf life

INTRODUCTION

Merguez is a raw sausage of North Africa origin widely consumed in different countries around the world either fried, barbecued or as an ingredient in some typical traditional dishes. In Morocco, it is usually prepared by butchers to be sold within two days because of its highly perishable nature. This shelf life may be shorter when merguez sausages are produced in poor hygienic conditions and exposed for sale at ambient temperature as is usually the case in the traditional butcher shops. Furthermore, merguez is a potential vehicle for serious food borne pathogens including *Listeria monocytogenes*, *Escherichia coli* O157 (Benkerroum *et al*., 2003; Benkerroum *et al*., 2004).

Many strategies have long been used to enhance the safety and keeping quality of meat products. Curing with a combination of sodium chloride and Nitrites salts is probably the oldest and the most widely used method for the preservation of meat products (Davidson and Harrison, 2002). However,

Corrssponding Author: Noreddine Benkerroum, Institut Agronomique et Vétérinaire Hassan II, Département des Sciences Alimentaires et Nutritionnelles, BP 6202, Instituts, 10101, Rabat, Morocco
Tel: +212 37 77 17 59 Fax: +212 37 77 81 35

the inability of nitrites to efficiently control some meat-born pathogen (Incze, 1998; Cleveland *et al*., 2001; Garriga *et al*., 2002) in addition to their potential carcinogenicity (Hartman, 1983; Wary and Sharan, 1991) have stimulated the interest of researchers and meat producers to search for alternative means to minimize or avoid their use in meat preservation. Therefore, the efficacy of natural food grade additives such as bacteriocins (Scannell *et al*., 2000; Benkerroum *et al*., 2003; Guinane *et al*., 2005; Ghalfi *et al*., 2006), essential oils of plant origin (Skandamis and Nychas, 2001; Cutter 2000; Tsigarida and Nychas, 2001), organic acids or their salts (Osthold *et al*., 1984; Unda *et al*., 1990; Brinkmann, 2001), adjunct starter cultures (Lüke, 2000; Hugas and Montfort, 1997) alone or in combination with each other or with conventional treatments in controlling specific pathogens and/or extending the shelf life of meat products has been investigated according to the multiple hurdles technology (Leistner, 1996).

The present study aimed to evaluate the effectiveness of a commercial mixture of sodium lactate and sodium acetate (9:1) to enhance the hygienic quality and the shelf life of merguez sausages.

MATERIALS AND METHODS

Sausage Preparation

Acetolac™, a commercial organic acid preparation consisting of a mixture of sodium lactate (90%) and sodium acetate (10%) was added at different concentrations to the butter mixture for merguez sausages making. The butter mixture contained: lean beef, 80%; fat beef, 20%; sugar, 5 (g kg^{-1}); salt, 18 (g kg^{-1}); red pepper, 10 (g kg^{-1}); cumin, 5 (g kg^{-1}); black pepper, 5 (g kg^{-1}); ginger, 5 (g kg^{-1}); olive oil (10 mL). The meat lean and the fat were cut up with a Kilia cutter (Germany) then mixed to the other ingredients. Five trials were conducted simultaneously. The same basal formulation described above was used for all trials; however, acetolac™ was added to four test trials at a concentration of 5, 10, 15 or 20 g kg^{-1}. No Acetolac™ was added to one trial to serve as a control. The sausage mixture of each trial was stuffed into natural casing (bovine small intestine) and stored in the refrigerator at approximately 8°C.

Sampling and Analyses

Samples (80 g of intact merguez segments) were withdrawn from each trial at regular intervals (0, 3, 7, 15 days) for sensory, chemical and bacteriological analyses. The merguez segments were aseptically cut with a knife into small pieces of about 0.5 cm each and kept in a sterile glass container for analysis.

Sensory Evaluation

The sensory evaluation was conducted by a trained sensory panel of four persons who were asked to judge whether or not samples were altered on the basis of the physical appearance and the smell.

Chemical Analysis

The pH was measured using an E250 pH-meter (Metrhom Ltd., Heriseau, Switzerland) on a 10 g sample minced and homogenized to 90 mL of distilled water. Total Volatile Basic nitrogen (TVB-N) was determined according to the micro-diffusion test of Conway (Conway, 1958)

Bacteriological Enumerations

A 25 g sample was pre-comminuted and mixed to 225 mL of a peptone solution with an Ultra Turrax (IKA Type T25, Jante and Kunkel, Germany) under aseptic conditions. The suspension was then decimally diluted and bacterial enumerations were carried out by the standard pour-plate technique or by the Most Probable Number (MPN) as appropriate. All media used were purchased

from Biokar (France). The total Aerobic Count (TAC) was carried out on plate count agar (BK 098) after incubation at 30°C for 48 h; coliforms were enumerated on Desoxycholate Citrate Lactose agar (DCL) after incubation at 37°C for 24 h; Fecal streptococci counts were determined by the MPN using Rothe broth (BK 060) and Litsky broth (BK 061) as the presumptive and confirmatory media, respectively and in each step the incubation was carried out at 37°C for 24 h; *Staphylococcus aureus* was enumerated on Baird Parker agar (BK 097) after incubation at 37°C for 24 h and the spores of sulphite reducing clostridia on meat liver agar (BK 097) after incubation at 44°C for 72 h.

Statistical Analysis

Each trial was repeated twice and each determination was done in duplicate. Statistical analysis was done by analysis of variance $\alpha = 0.05\%$ and Student t-test.

RESULTS AND DISCUSSION

Figure 1 summarizes the results of the trend of the pH and AVB-N in merguez samples during storage at 8°C. The results show that the pH has increased in all samples with a concomitant increase in the TVB-N content (Fig. 2) indicating that the pH increase resulted from a proteolytic activity that took place in merguez during conservation at refrigeration temperature. Such an activity may result from microbial proteases mainly produced by psychrotrophic bacteria (e.g., *Pseudomonas* sp., *Proteus* sp. or other species of the Enterobacteriaceae family) known to be the main spoilage microorganisms in meat products or by indigenous proteases naturally occurring in meat and in the intestine used as a casing to portion the sausages. The Fig. 1 and 2 show also that the increase in pH

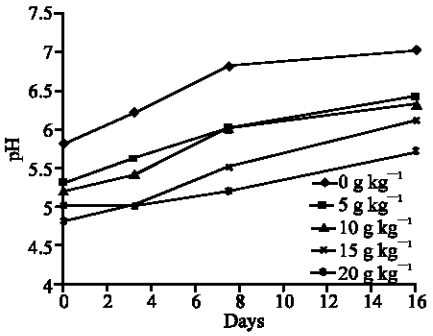

Fig. 1: pH evolution during storage of merguez sausages treated with different levels of organic acids

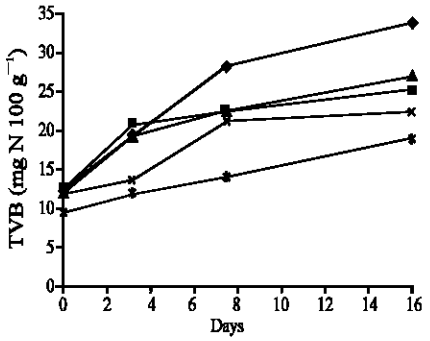

Fig. 2: Total Volatile Basic Nitrogen (TVB) evolution during storage of merguez sausages treated with different levels of organic acids. Symbols are as in Fig. 1

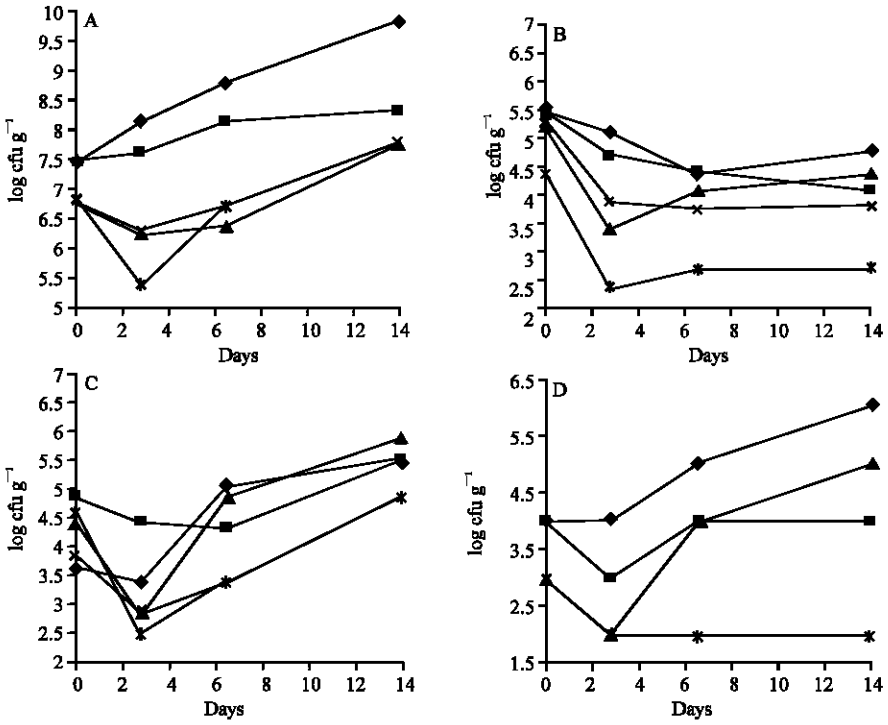

Fig. 3: Bacterial counts evolution as function of time in merguez sausages treated with organic acids (Acetolac™) at different concentrations: Total aerobic count (A), Coliforms (B), Fecal streptococci (C) and *Staphylococcus aureus* (D). Symbols are the same as in Fig. 1. The counts of S. aureus in samples treated with 15 g kg^{-1} were the same as in those treated with 5 g kg^{-1} in D

and TVB-N was significantly slower in presence of acetolac™ and that such an effect was more pronounced as the concentration of added Acetolac™ increased. At the highest concentration of added Acetolac™ (2% w/w), no significant ($p>0.05$) changes (increase or decrease) in the pH were noted during the first 7 days of cold storage (Fig. 1). The fact that no decrease in the pH was observed suggests that the acidifying bacteria naturally occurring in the sausages were also inhibited by the organic acid mixture resulting in a stabilisation of the pH during this period. It is worthwhile to mention that the acidification of merguez sausages during storage is also regarded as a sensory alteration by consumers.

Figure 3 showes the evolution of the viable counts of various bacterial groups of health or spoilage significance as function of time during storage at 8°C. A dramatic decrease in the colony forming units (cfu) of all of the bacterial groups enumerated was recorded within the first three days of storage except for the TAC in the control samples and in samples treated with 5 g kg^{-1} where the cfus continue to increase. However, after the third day a significant increase ($p<0.05$) in the counts of all the bacterial groups was observed, with the exception of *Staphylococcus aureus* (Fig. 3D) whose counts remained practically constant throughout the whole period of storage after reaching a minimum level at day 3. Growth re-initiation of microbial groups after the third day of storage for all samples may be explained either by the pH increase or by the depletion of the organic acids used as a carbon and energy source by some microorganisms (e.g., yeasts and moulds). Adaptation of microorgnaisms to antimicrobial agents upon extended exposure has also been reported to account for such a rebound phenomenon

(Davidson and Harrison, 2002). The counts of sulphite reducing clostridia were below the detectable limit of 10 cfu g^{-1}, which is a good indication regarding the safety of the product, as the presence of pathogenic clostridia have long been know to represent a major health risk associated with the consumption of meat products.

As for the keeping quality of merguez sausages, the sensory analysis revealed that the growth of molds, surface mucoidness or discoloration, off-odours and other perceivable alterations were delayed by at least 5 days in samples with added organic acids at the concentrations ranging between 10 and 20% (w/w) as compared to control samples (without added Acetolac™). The latter samples were altered within 5 days of storage. Therefore, the treatment of merguez sausages with Acetolac™ has extended the shelf life of the sausages by five days at the refrigeration temperature which is regarded as very significant considering the highly perishable character of merguez.

CONCLUSIONS

The results of the present study suggest that the addition of Acetolac™ at levels ranging between 10 and 20 g kg^{-1} of butter mixture appears to effectively enhance the hygienic quality Merguez sausages and extend its shelf life by 5 days at refrigeration temperature. The use of Acetolac™ in Merguez preservation is even more appropriate as the organic acids that it contains are safe to consumers. However, this measure does not represent a substitute for the scrupulous application of the good manufacture practices, but rather add up a safety factor.

ACKNOWLEDGMENTS

The authors are grateful to the Société Anonyme des Palmeraies Koutoubia, SAPAK (Mohammedia Morocco) for supporting part of the present research.

REFERENCES

Benkerroum, N., A. Daoudi and M. Kamal, 2003. Behavior of a *Listeria monocytogenes* in a raw sausage (merguez) in presence of a bacteriocin-producing strain as a protective culture. Meat Sci., 63: 53-58.

Benkerroum, N., Y. Bouhlal, A. El Attar and A. Marhaben, 2004. Occurrence of shiga toxin-producing *Escherichia coli* O157 in selected dairy and meat products marketed in the city of Rabat, Morocco. J. Food Prot., 67: 1234-1237.

Brinkmann, W., 2001. Haltbarkeit und sicherheit verbessert. Milchsäure und ihre salze entfalten positive effekte auf fleischwaren. Fleischwirtsch, 6: 26-29.

Cleveland, J., T.J. Montville, I.F. Nes and M.L. Chikindas, 2001. Bacteriocins: Safe, natural antimicrobials for food preservation. Int. J. Food Microbiol., 71: 1-20.

Conway, E.J., 1958. Microdiffusion Analysis and Volumetric Error. The Mac Millan Compagny, New York, NY, pp: 199.

Cutter, C.N., 2000. Antimicrobial effect of herb extracts against *Escherichia coli* O157:H7, *Listeria monocytogenes* and *salmonella typhimurium* associated with beef. J. Food Prot., 63: 601-607.

Davidson, P.M. and M.A. Harrison, 2002). Resistance and adaptation to food antimicrobials, sanitizers and other process controls. Food Technol., 56: 60-78.

Garriga, M., M.T. Aymerich, S. Costa, J.M. Monfort and M. Hugas, 2002. Bactericidal synergism through bacteriocins and high pressure in a meat model system during storage. Food Microbiol., 19: 509-518.

Ghalfi, H., P. Kouakou, M. Duroy, A. Daoudi, N. Benkerroum and P. Thonart, 2006. Application of an antilisterial bacteriocin-producing strain of *Lactobacillus curvatus* CWBI-B28 as a protective culture in bacon meat and effect of fat and sodium nitrites on bacteriocin production and activity. Food Sci. Technol. Int., 12: 325-333.

Guinane, C.M., P.D. Cotter, C. Hill and R.P. Ross, 2005. Microbial solutions to microbial problems; lactococcal bacteriocins for the control of undesirable biota in food. J. Applied Microbiol., 98: 1316-1325.

Hartman, P.E., 1983. Review: Putative mutagens and carcinogens in foods. I. Nitrate/nitrite ingestion and gastric cancer mortality. Environ. Mut., 5: 111-121.

Hugas, M. and J.M. Monfort, 1997. Bacterial starter cultures for meat fermentation. Food Chem., 59: 547-554.

Incze, K., 1998. Dry fermented sausages. Meat Sci., 49: 169-177.

Leistner, L., 1996. Food protection by hurdle technology. J. Food Prot., 2: 2-27.

Lüke, F.K., 2000. Utilization of microbes to process and preserve meat. Meat Sci., 56: 105-115.

Osthold, W., H.K. Shin, J. Dressel and L. Leistner, 1984. Improving the storage life of carcasse by treating their surfaces with an acid spray. Fleischwirtsch, 64: 828-830.

Scannell, A.G.M., R.P. Ross, C. Hill and E.K. Arendt, 2000. An effective lacticin biopreservative in fresh pork sausage. J. Food Prot., 63: 370-375.

Skandamis, P.N. and G.J.E. Nychas, 2001. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. J. Applied Microbiol., 91: 1011-1022.

Tsigarida, E. and G.J.E. Nychas, 2001. Ecophysiological attributes of a *Lactobacillus* sp. and a *Pseudomonas* sp. on sterile beef fillets in relation to storage temperature and film permeability. J. Applied Microbiol., 90: 696-705.

Unda, J.R., R.A. Molins and H.W. Walker, 1990. Microbiological and some physical and chemical changes in vacuum packaged beef streaks treated with combination of potassium sorbate, phosphate, sodium chloride and sodium acetate. J. Food Sci., 55: 323-326.

Wary, K.K. and R.N. Sharan, 1991. Cytotoxic and cytostatic effects of arecoline and sodium nitrite on human cells *in vitro*. Int. J. Cancer, 47: 396-400.