PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 5 (6): 601-605, 2006
ISSN 1680-5194

Microbial and Sensory Changes During the Cold Storage of Chicken Meat Treated with Bacteriocin from *L. brevis* OG1

S.T. Ogunbanwo[1]* and B.M. Okanlawon[2]
[1]Department of Botany and Microbiology, University of Ibadan, Nigeria
[2]Department of Biomedical Sciences, Ladoke Akintola University of Technology, Osogbo, Osun State, Nigeria

Abstract: The influence of Bacteriocin produced by *L. brevis* OG1 on safety and sensory analysis of chicken tissue immobilized in edible film and stored at 4°C were assessed. Immobilization of Bacteriocin in gel and application to the surface of lean tissue of broiler chicken was effective for reducing microbial load up to 21 days at 4°C when compared with untreated chicken tissue. The microbial population of untreated and alginate - treated chicken tissue grew to greater than 8 $\log_{10}$ after 21days under refrigerated conditions while that of Bacteriocin treated and alginate - Bacteriocin treated samples of chicken tissue was less than 6 $\log_{10}$ after 21 days. Microbial counts in post - rigor tissue treatments were greater than that obtained from pre - rigor tissue treatments. Chicken lean tissue treated with Bacteriocin - alginate solution has the best sensory attributes in terms of appearance and odour. This indicated that immobilization of bacteriocin in edible film could extend shelf - life of chicken up to 14 days during refrigerated storage without adversely affecting the odour, and appearance of the chicken and increased safety of the chicken for human consumption.

Key words: Bacteriocin, *Lactobacillus brevis* OG1, Chicken meat, calcium alginate, Shelf-life

Introduction

Chicken meat products are economically important refrigerated products with high consumption in European and African countries. It provides much of the protein intake of man all over the world. (Lee *et al*., 1971). However, due to lack of storage facilities and adequate processing procedure, availability of animal proteins to the populace especially in the developing countries is becoming a mirage (Kung, 1990).

Shelf - life of refrigerated fresh muscle foods is determined mainly by microbiological and physical qualities during storage and handling (Chang *et al*., 1999). Reduced product quality results in reduced consumer acceptance (Kotula and Pandya, 1995; Lee *et al*., 1996). Decontamination of fresh meat using chemical agents depends on concentration, type, exposure times and consumer acceptance (Kim *et al*., 1995; MarShall and Kim, 1996). Sulphur dioxide in the form of sodium metabisulphite, is used to inhibit microbial spoilage of fresh beef in storage. However sulphites have been linked to the aggravation of asthmatic and other respiratory conditions, urticaria, angioedema, headache and gastro-intestinal dysfunction in both sulphate - sensitive and normal individuals (Simon, 1990).

Based on these facts, there is need for an alternative method that may provide additional safety and shelf - life measures for raw meat products.

Biopreservation has gained increasing attention as a means of naturally controlling the Shelf - life and safety of meat products. Some lactic acid Bacteria (LAB), among those commonly associated with meats, demonstrate antagonism towards pathogenic and spoilage organisms (Lucke, 2000; Vermeiren *et al*., 2004). Nisin is perhaps one of the best examples of a natural preservative produced by some strains of *Lactococcus lactis* and has been proven to be non - toxic (Frazer *et al*., 1962; Shtenberg and Ignatey, 1970).

However, there are limited studies on the effect of other bacteriocins produced by other LAB, for use on refrigerated chicken, to extend its shelf - life. In this study, quality attributes and shelf - life of refrigerated chicken treated with bacteriocin produced by *L. brevis* OG1 was investigated.

Materials and Methods

Microorganism: The *Salmonella kentucky* (ATI) strain employed in this work was obtained from the culture collection of the veterinary Research Institute, Vom Jos, Nigeria. It was originally isolated from raw chicken. The organism was grown in brain heart infusion broth for 24 h at 37°C.

Lactobacillus *brevis* OG1, which had earlier been found to produced bacteriocin, that have inhibitory activity on *Salmonella Kentucky* ATI, *Listeria monocytogenes*, etc (Ogunbanwo *et al*., 2003) was chosen for this experiment.

Bacteriocin preparation: *Lactobacillus brevis* OG1 was propagated in MRS broth (pH 5.5) with reduced concentration of glucose (0.25% w/v) and peptone (0.5% w/v) for 72h at 30°C in an anaerobic jar. Extraction of

Table 1: Microbial load (Log_{10} cfu/g) of pre-rigor chicken stored at 4°C for 21 days

	Storage time (days)				
Treatment	0	1	7	14	21
Untreated (control)	3.883[a]	4.283[a]	5.400[a]	6.583[a]	8.133[a]
Alginate	3.6[a]	4.017[a]	5.717[a]	6.817[a]	8.083[a]
Bacteriocin	1.983[b]	1.733[b]	2.833[b]	4.883[b]	6.100[b]
Bacteriocin - alginate	0.983[c]	0.733[c]	1.883[c]	2.567[c]	3.733[c]

Means values in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P<0.05$).

bacteriocin was carried out using the method of Schillinger and Lucke (1989). Inhibitory activity from hydrogen peroxide was eliminated by the addition of 5mg/ml catalase (C-100 bovine liver, sigma) (Daba *et al*., 1991). The culture supernatant was purified according to the method of Ogunbanwo *et al.* (2003). Antagonist activity against *Salmonella* kentucky AT1 was determined using a well diffusion assay (Schillinger and Lucke, 1989). The antimicrobial activity of the bacteriocin was defined as the reciprocal of the highest dilution showing inhibition of the indicator lawn and was expressed in activity units per ml (AU/ml).

Chicken preparation and treatments: Lean tissue (cutaneous trunci) from the outer surfaces of pre-rigor (10min post exsanguinations) chicken carcasses was obtained from a local poultry processing plant and stored in insulated carriers to prevent rapid cooling. They were transported to the laboratory and used within 3h of slaughter. Prior to this, the cutaneous trunci was trimmed to fit onto sterile trays. Also post – rigor frozen (24h post mortem) and thawed lean tissue (cutaneous trunci) from chicken carcasses was weighed to obtain 100g with surface area of 10cm x 10cm x 0.5cm, for both pre – rigor and post – rigor tissue, the surfaces were surface sterilized by U.V light (Cutter and Siragusa, 1996). Sterility was monitored by individual sampling of pieces of uninoculated tissue using the enumeration procedures described below.

Over night cultures of *S. kentucky* AT1 were diluted 1:1000 in sterile peptone water (pH 7.0) to obtain a viable cell population of approximately 5.0×10^6 cfu/ml. The inoculum was aseptically sprayed onto the tissue with a hand - held sprayer and left undisturbed for 20min, at 26°C, prior to applying treatment. Initial bacterial populations of approximately 3.8×10^4 cfu/g were obtained using this procedure. Lean tissues inoculated with *S. kentucky* AT1 were separated into four batches and treated as follows: untreated (U); treated with 20ml of alginate solution (1% w/ v sodium alginate sigma pH 7.0) and cross linked with 20ml of $CaCl_2$ (pH 7.0) (A); treated with 20ml of purified bacteriocin (3200 AU/ml) from *L. brevis* OG1 (B) ; treated with 20ml of alginate solution containing purified bacteriocin (3200 AU/ml) and cross linked with 20ml of $CaCl_2$ (AB). In experiments U, A, B, and AB, treatment were performed at 26°C by spraying the compound (10ml) evenly over the tissues with a sterile hand - held spray bottle and allowed to remain undisturbed for 20 min. Chicken was collected in a sterile polyethylene stomacher bag, tied off and stored at 4°C until sampled. At 0 (within 20 min of grinding), 7, 14, or 21 days of refrigeration, 25g samples were taken from each batch of treated chicken. A grinder (Moulinex Model 278 France) was used to grind individual batches of treated chicken tissues. All individual samples were there after ground for 2 min in stomacher bag containing 25 ml of buffered peptone water (pH 7.0). Each ground sample was serially diluted in buffered peptone water (pH 7.0) and plated in duplicate on plate count agar. The plates were incubated at 37°C for 2 days and microbial load was determined.

Sensory evaluation: Sensory evaluation of both post-rigor and pre-rigor chicken lean tissue under various treatments was performed by a 20 - member untrained panel. Odour and appearance of treated uncooked chicken (Lean tissue) were evaluated during storage at 4°C by sampling every 7 days. Treated chicken (lean tissue) were judged against fresh chicken (control) which were assigned a score a nine - point Hedonic scale ranging from nine, like extremely to one dislike extremely.

Statistical analyses: Microbial populations were converted to Log_{10} cfu/g. Least squared means (LSM) of microbial population (Log_{10} cfu/g) were calculated from six experimental replications for each experiment. Analysis of variance (ANOVA) was performed for both microbial and sensory evaluation using the General Linear Model (SAS, 1996). Inoculum's counts were used as a co - variant to normalize data between treatment replications. Statistical significance was defined as $P \leq 0.05$, unless otherwise stated.

Results

Evaluation of the efficiency of bacteriocin produced by *L. brevis* OG1 on the safety and sensory analysis of chicken immobilized in edible film was carried out. It was observed that immobilization of bacteriocin produced by *L. brevis* OG1 in calcium alginate gel and

Table 2: Microbial load (Log_{10} cfu/g) of post-rigor chicken stored at 4°C for 21 days

	Storage time (days)				
Treatment	0	1	7	14	21
Untreated (control)	4.467[a]	4.867[a]	6.250[a]	7.650[a]	8.335[a]
Alginate	4.367[a]	4.683[a]	6.517[a]	7.817[a]	8.633[a]
Bacteriocin	2.383[b]	2.200[b]	3.733[b]	5.450[b]	6.100[b]
Bacteriocin - alginate	1.283[c]	1.200[c]	2.600[c]	3.467[c]	4.367[c]

Means values in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P<0.05$).

application to the surface of lean tissue (cutaneous trunci) of broiler chicken was effective for reducing microbial load up to 21 days at 4°C when compared with untreated (control) chicken tissue. The interactions of tissue by day and treatment by day using ANOVA, demonstrated significant different ($P<0.001$) in microbial population due to the effect of tissue, treatment and day. Microbial populations on the pre - rigor and post - rigor broiler chicken tissue left untreated (U) and following treatments with alginate (A), bacteriocin (B) and alginate solutions containing bacteriocin (AB) are shown in Table1 and 2 respectively.

Microbial load from alginate treated pre-rigor and post-rigor chicken tissues were not statistically different from untreated tissue. Untreated post - rigor chicken have microbial load of 6.250 Log_{10} cfu/g at day 7 while alginate treated post - rigor chicken have 6.517 Log_{10} cfu/g at the same day (Table 2). However, the microbial populations of untreated tissue and alginate treated tissue grew to greater than 8 Log_{10} cfu/g of chicken after 21 days under refrigerated conditions while that of Bacteriocin treated and alginate - Bacteriocin treated samples of chicken tissue was less than 6 $Log/_{10}$ cfu/g after 21 days under refrigerated conditions in both pre-rigor and post rigor chicken.

Microbial populations from chicken tissues treated with alginate solution containing bacteriocin in both pre-rigor and post-rigor chicken tissues were statistically different from those treated with bacteriocin only ($P<0.05$). Ultimately, microbial counts in post-rigor chicken tissue treatments (Table 2) were greater than that obtained from pre-rigor tissue treatments (Table 1).

Sensory analysis indicated that untreated chicken at day 0 (within 3 hours of slaughter) has the best sensory attributes and scored 9.0 when compared with all other treated chicken at the same time. After day 7, chicken treated with bacteriocin alone and bacteriocin-alginate solution has the best sensory attributes in terms of appearance and odour compared with untreated and alginate treated chicken. Although, as the storage time increases the level of acceptability of the sensory attributes decreases in all the treated and untreated chicken. There were significant difference in sensory attributes of chicken treated with bacteriocin alone and bacteriocin - alginate solution at 14 and 21 days of storage (Table 3 and 4). Post-rigor chicken treated with bacteriocin alone had 4.2±0.11 odour score at day 21 of storage while post-rigor chicken treated with alginate solutions containing bacteriocins had 5.0±0.02 odour score at the same time of storage.

Discussion

The preservation effect of bacteriocin produced by *L. brevis* OG1 was evaluated for chicken immobilized in edible films. *L brevis* OG1 produce bacteriocin that has a broad spectrum of inhibition against pathogens and food spoilage organisms (Ogunbanwo *et al.*, 2003).

In this study, it was observed that immobilization of bacteriocin produced by *L. brevis* OG1 in calcium alginate gel and application to the surfaces of lean tissue (cutaneous) of broiler chicken was effective for reducing microbial load up to 21 days of refrigerated storage. Broiler chicken is a raw comminuted fresh meat product, which provides a suitable environment for proliferation of meat spoilage microorganisms (Gill, 1979) and common food pathogens, including *Salmonella species* and *Staphylococcus aureus* (Farber *et al.*, 1988). This could be the reason why microbial load of untreated and alginate treated chicken grew to greater than 8 log_{10} cfu/g after 21 days under refrigerated conditions. Preservation of fresh meat involves the use of sodium metabosulphite, but as this preservative is associated with undesirable side effects (Simon, 1990), a replacement is required which does not influence the quality and organoleptic attributes of the product. The preservative effect of bacteriocin used in this study has been incorporated into meat systems by a number of workers (Cutter and Siragusa, 1996; Scannell *et al.*, 1997). A possible explanation for the reduction in number of microorganisms on bacteriocin and bacteriocin – alginate treated chicken, may be that the cell wall of the organisms was damaged by either osmotic or cold shock or a combined effect of the two, allowing the penetration of bacteriocin into the cell membrane of the microorganisms (Harris *et al.*, 1992). The data from the present study demonstrated that immobilization of bacteriocin in calcium-alginate gel and application to the surface of lean tissue of broiler chicken is more effective for reducing microbial populations than applying bacteriocins directly. Siragusa

Table 3: Sensory attributes of pre – rigor chicken stored at 4^0C for 21 days

	Storage time (days)							
	Appearance score				Odour scoreTreatment			
	0	7	14	21	0	7	14	21
Untreated (control)	9.0±0.03^a	6.0±0.02^a	4.0±0.01^a	3.0±0.01^a	9.0±0.02^a	5.0±0.05^a	4.0±0.02^a	2.0±0.10^a
Alginate	7.6±0.02^b	5.9±0.01^a	3.6±0.04^a	2.8±0.18^a	7.0±0.01^b	5.2±0.02^a	4.0±0.01^a	2.0±0.02^a
Bacteriocin	8.6±0.06^a	7.6±0.02^b	5.0±0.01^b	4.6±0.02^b	8.6±0.01^a	6.0±0.02^b	5.0±0.01^b	3.5±0.01^b
Bacteriocin - alginate	8.6±0.04^a	8.0±0.01^b	6.5±0.03^c	5.3±0.02^c	8.6±0.03^a	7.5±0.01^b	6.0±0.01^c	4.5±0.02^c

Means values in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P<0.05). Rejected = 0-4.9; Accepted = 5.0-10.0

Table 4: Sensory attributes of post– rigor chicken stored at 4^0C for 21 days

	Storage time (days)							
	Appearance score				Odour scoreTreatment			
	0	7	14	21	0	7	14	21
Untreated (control)	9.0±0.02^a	6.6±0.03^a	4.5±0.04^a	3.0±0.02^a	9.0±0.11^a	6.5±0.03^a	4.2±0.04^a	2.8±0.14^a
Alginate	8.0±0.04^b	7.0±0.03^a	4.5±0.02^a	3.0±0.01^a	8.0±0.04^b	6.3±0.03^a	4.0±0.01^a	2.5±0.10^a
Bacteriocin	8.6±0.01^a	8.0±0.02^b	6.0±0.01^b	5.0±0.04^b	8.6±0.01^a	7.5±0.03^b	5.5±0.12^b	4.2±0.11^b
Bacteriocin – alginate	8.6±0.02^a	8.0±0.03^b	7.2±0.01^c	6.0±0.02^c	8.6±0.03^a	8.0±0.01^b	6.5±0.05^c	5.0±0.02^c

Means values in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P<0.05). Rejected = 0- 4.9; Accepted = 5.0-10.0

and Dickson (1992; 1993) have demonstrated that bacterial reduction were greater following immobilization of antimicrobial compounds (e.g organic acids) in calcium alginate gels than when the compounds were applied alone. The results obtained in this study indicated that the use of bacteriocin alone or bacteriocin immobilized in calcium alginate gives increased protection against Salmonella species, a longer product shelf life and provides a promising alternative to sulphate in fresh meat. Since consumers nowadays, demand food products with fewer synthetic additives (Daeschel, 1993) but increased safety, quality and shelf life. These demands have led to renewed interest in the use of natural antimicrobials to preserve foods. The application of bacteriocin produced by lactic acid bacteria is gaining interest. Some LAB show special promise as they do not pose any health risk to man and are able to prevent the growth of undesirable bacteria and opportunistic pathogens such as *Staphylococcus aureus*, *Listeria monocytogens* and Salmonella *species*.

Acknowledgements

The authors are grateful for the technical assistance of Prof A.I. Sanni, Department of Botany and Microbiology, University of Ibadan, Nigeria.

References

Chang, D.E., H.C. Jung, J.S. Rhee and J.G. Pan, 1999. Homofermentative production of D - or L - lactate in metabolically engineered *E. coli* RR Appl. and Environ. Microbiol., 65: 1384-1389.

Cutter, C.N. and G.R. Siragusa, 1996. Reduction of Brochrothrix thermosphacta on beef surfaces following immobilization of nisin in calcium alginate gels. Letters in Appl. Microbial., 23: 9-12.

Daba, H., S. Pandian, J.F. Gosselin, R.E. Simard, J. Hwang and C. Lacroix, 1991. Detection and activity of bacteriocin produced by *Leuc. Mesenteriodes*. Appl. and Environ. Microbiol., 57: 3450-3455.

Daeschel, M.M., 1993. Antimicrobial substance from lactic acid bacteria for use as food preservatives. Food Tec., 43: 164-167.

Farber, J.M., S.A. Mlacom, K.F. Weiss and M.A. Johnson, 1988. Microbiological quality of fresh and frozen breakfast type sausages sold in Canada. J. Food Protec., 51: 397-401.

Frazer, A.C., M. Sharratt and J.R. Hickman, 1962. The biological effects of food additives. I. Nisin. J. Sci. Food and Agri., 13: 32-41.

Gill, C.O., 1979. A review, intrinsic bacteria in meat. J. Appl. Bacteriol., 367-378.

Harrris, L.J., H.P. Fleming and T.R. Klaehammer, 1992. Developments in nisin research. Food Res. Int., 25: 57-66.

Kim, C.R., J.O. Hearnsberger, A.P. Vickery, C.H. White and D.L. MarShall, 1995. Extending shelf life of refrigerated catfish fillets using sodium acetate and monopotassium phosphate. J. Food Protec., 58: 644- 647.

Kotula, K.L. and Y. Pandya, 1995. Bacterial contamination of broiler chickens before scalding. J. Food Protec., 58: 1326-1329.

Kung, L. Proc, 1990. Florida Ruminant Symposium. In Onilude, A.A. 1999. Fermented poultry litter and fungal enzyme supplementation of high-fibre broiler diets: Growth responses, carcass characteristics and blood of feed chicks. Nabrung, 43: 54-60.

Lee, C., J.M. Howe, K. Carlson and H.E. Clark, 1971. Nitrogen retention of young men fed rice with or without supplementary chicken. Am. J. Clin. Nutr., 24: 318-323.

Lee, J.I., E.H. Shin, C.R. Kim and K.H. Kim, 1996. Reducing microbial populations on refrigerated pork harms treated with acetic acid. Kr. J. Food Nutr., 9: 484.

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

Marshall, D.L. and C.R. Kim, 1996. Microbiological and Sensory analyses of Refrigerated Catfish fillets treated with acetic and lactic acid. J. Food Quality, 19: 317-329.

Ogunbanwo, S.T., A.I. Sanni and A.A. Onilude, 2003. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. Afr J. Biotec., 2: 219-227.

SAS, 1996. SAS users Guide: Statistic Cary, NC: SAS Institute Inc.

Schillinger, V. and F.K. Lucke, 1989. Antibacterial activity of *L. Sake* isolated from meat. Appl. Environ. Microbiol., 55: 1901-1906.

Scannell, A.G.M., C. Hill, D.J. Buckley and E.K. Arendt, 1997. Determination of the influence of organic acids and nisin on shelf – life and microbiological safety aspects of fresh pork sausage. J. Appl. Microbiol., 83: 407-412.

Shtenberg, A.J and A.D. Ignatey, 1970. Toxicological evaluation of some combinations of food preservatives. Food and Cosmetics Toxicol., 8: 369-380.

Simon, R.A., 1990. Adverse effects of sulphur dioxide, sulphites and wine in proceedings of the seventh Australian wine Industry Technological Conference pp. 210-212.

Siragusa, G.R. and J.S. Dickson, 1993. Inhibition of *Listeria manocytogenes, Salmonella* typhimurium and *Escherichia coli* 0157: H7 on beef muscle tissue by lactic or acetic acid contained in calcium alginate gels. J. Food Safety, 13: 147-158.

Siragusa, G.R. and J.S. Dickson, 1992. Inhibition of *Listeria monocytogenes* on beef tissues by application, of organic acid immobilized in a calcium alginate gel. J. Food Sci., 57: 293-296.

Siragusa, G.R. and J.S. Dickson, 1993. Inhibition of *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* 0157: H7 on beef muscle tissue by lactic or acetic acid contained in calcium alginate gels. J. Food Safety, 13: 147-158.

Vermeiren, L., F. Devlieghere and J. Debevere, 2004. Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. Int. J. Food Microbiol., 96: 149-164.