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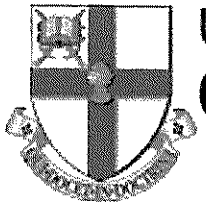
This certificate is awarded to

UGUR GOGUS

"The effects of nisin, oil-wax coating and youghurt on the quality of refrigerated chicken meat".

in recognition of valuable contributions to

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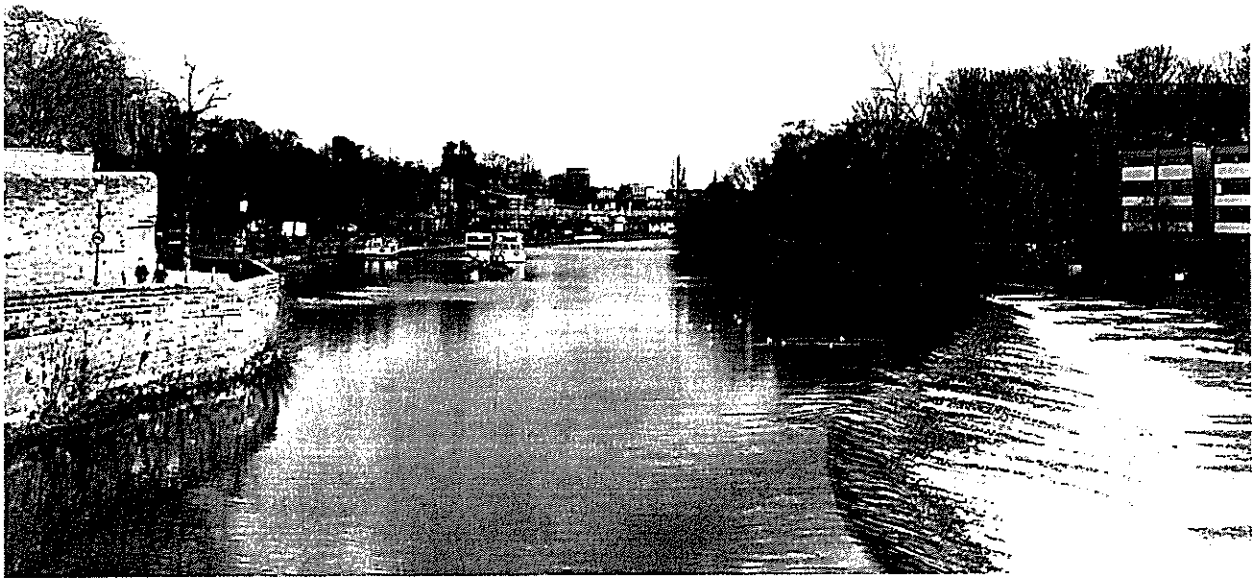
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Date 10th April, 2007

Programme, abstracts and list of delegates

1st International Chester Food Science & Technology CONFERENCE

10TH – 13TH April 2007



Organised by
Society for Food and Agricultural Immunology, the Chester centre for science communication and The University of Chester.

Society for Food and Agricultural Immunology

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11.05	L-03	F. D. Baah, B. Maziya-Dixon, R. Asiedu, I. Oduro & W. O. Ellis. "Evaluation of Diosorea alata varieties for making pounded yam".
11.25	L-04	Ali Asghar, Faqir Muhammad Anjum, Jonathan C. Allen, Christopher R. Daubert & Sharon R. Ramsey. "Effect of dairy byproduct proteins as a functional ingredient on fundamental rheological properties of frozen wheat flour doughs".
11.45	L-05	D Jaros, M Jacob, S Nöbel, C Heidig, C Partschefeld and H Rohm "Gels from Enzymatically Modified Milks: Impact of Acidification Agent on Their Physical and Rheological Properties".
12.15 - 13.15		Lunch: to take place in the main dining hall
13.15 - 14.45		**1st Poster session**
		Session 2 - Processing Technologies **Chair: Dr. Niall Young**
14.50	PL-02	**2nd Plenary lecture: Dr Saphwan Al-Assaf** "Correlation of hydrocolloids characteristics with functional properties".
15.30	L-06	Arthur Zdunek "Determination of apple texture using acoustic emission".
15.50 - 16.20		Coffee Break
16.25	L-07	Takahiro Funami "AFM imaging of food polysaccharides in relation to their rheological properties".
17.05	L-08	Ugur Gogus, Faruk Bozoglu & Seyhun Yardugul" The effects of nisin, oil-wax coating and youghurt on the quality of refrigerated chicken meat".
17.25	L-09	Seyed H. HosseiniParvar, J. Keramat, M. Kadivar, Seyed M. A. Razavi & E. Khanipour. "Optimizing conditions for enzymatic extraction of edible gelatin from the cattle bones using response surface methodology".
18.30		Dinner: to take place in the main dining hall

Thursday 12th April

L-08

THE EFFECTS OF NISIN, OIL–WAX COATING AND YOGURT ON THE QUALITY OF REFRIGERATED CHICKEN MEAT.

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Evisceration makes chicken carcasses susceptible to microbial contamination from gastrointestinal sources. A preventive action between spray washing and air chilling, a yogurt dip, may eliminate concerned contamination and enhance the bactericidal effect of nisin on *Salmonella*. This study was designed to determine whether synergism occurred with or without the application of an oil–beeswax coating. A 5 h yogurt dip then nisin with coating' and a 5 h yogurt dip then nisin, indicated significant inhibition of mesophilic aerobic bacteria and *Salmonella* with 2.11 and 1.97 log reductions, supported by pH, chromatographic and sensory findings ($p<0.01$).

Author Keywords: Chicken; *Salmonella typhimurium; Pseudomonas*; MAB; Yogurt; Critical point; Nisin; Coating

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The effects of nisin, oil–wax coating and yogurt on the quality of refrigerated chicken meat

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Received 2 February 2003; received in revised form 22 August 2003; accepted 26 August 2003

Abstract

Evisceration makes chicken carcasses susceptible to microbial contamination from gastrointestinal sources. A preventive action between spray washing and air chilling, a yogurt dip, may eliminate concerned contamination and enhance the bactericidal effect of nisin on *Salmonella*. This study was designed to determine whether synergism occurred with or without the application of an oil–beeswax coating. A 5 h yogurt dip then nisin with coating' and a 5 h yogurt dip then nisin, indicated significant inhibition of mesophilic aerobic bacteria and *Salmonella* with 2.11 and 1.97 log reductions, supported by pH, chromatographic and sensory findings ($p < 0.01$).
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Keywords: Chicken; *Salmonella typhimurium*; *Pseudomonas*; MAB; Yogurt; Critical point; Nisin; Coating

1. Introduction

During post-evisceration, poultry is highly susceptible to microbial contamination due to the evisceration process itself. Pre-evisceration treatments from stunning-slaughter to scalding (Fig. 1) can be effective in reducing microbial contamination. For example the bactericidal action of heat during scalding and the application of various substances such as organic acids and chlorine (Varnam & Sutherland, 1995). Nevertheless, carcasses receive only a spray washing after evisceration which brings about no more than 1 log reduction in microbial numbers and may be of importance in removal of *Salmonella*. In the case of water chilling followed by spray washing, the chlorine added to the chiller tanks or similar bactericidal chemical applications can be destructive for *Salmonella* and other contamination flora (Goodfellow, 1995), but there is no similar application for the carcasses which are taken directly for air chilling. Since air chilling has no bactericidal effect on *Salmonella* survivors and contamination flora, the step between spray washing and air chilling should be defined as a critical control point (Fig. 1). The bactericidal effect, may result from nisin usage and acidity of yogurt, as alternatives to chlorine, organic acid and bactericidal solutions, could be preventative in this step. One of the main aims of this study was to observe whether this was a preferred alternative.

Salmonella typhimurium has been reported as one of the predominant Gram negative pathogenic bacteria in chicken meat causing serious food poisoning (Henderson, Bounus, & Lee, 1999). Nisin, a bacteriocin, produced by *Lactococcus lactis* inhibits many Gram positive food-borne pathogens namely *Listeria*, *Clostridium*, *Bacillus*, *Streptococcus* (Ogden & Tubb, 1985). Nettles and Siragusa (1994) indicated 1.8 log reduction in the Gram positive count of beef carcass surfaces followed by nisin spray treatment. Nevertheless, nisin is not widely used in poultry meat as its activity is limited to Gram positives. However, studies have revealed that the activity of nisin can be synergetically increased by combined use of other compounds such as organic acids, EDTA and ethyl alcohol that increase the rate of nisin penetration into Gram negative cell walls (Hauben et al., 1997; Sheffet, Sheldon, & Klaenhammer, 1995; Stevens, Sheldon, Klapes, & Klaenhammer, 1991). Similarly Gill and Holley (2000) observed 1 and 1.5 log reductions respectively of

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doi:10.1016/j.foodcont.2003.08.007

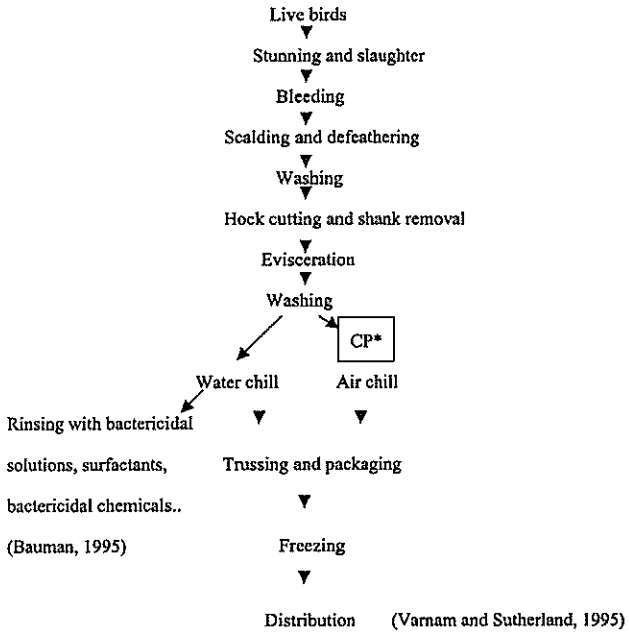


Fig. 1. The critical point between spray washing and air chilling in the flow diagram of the conventional processing of chickens, for which this study was performed. *: The point which should be concerned as critical point between spray washing and air chilling, susceptible to abdominal contamination.

Escherichia coli 0157:H7 and *S. typhimurium* on bologna and ham treated with lysozyme, nisin and EDTA. In another study, Pawar, Malik, Bhilegaonkar, and Barbuddhe (2002) increased the antilisterial effect of nisin on raw buffalo meat mince with 2% sodium chloride. Nattress, Yost, and Baker (2001) pointed out the synergistic effect of lysozyme with nisin to control meat spoilage. Another application to enhance the effect of nisin on Gram negative bacteria was hydrostatic pressure. High hydrostatic pressure as in combination with lysozyme and nisin, appeared as an attractive process for meat products to avoid post-processing contamination by increasing the death rate of living cells because of the lethal injuries to bacterial cell membranes (Garriga, Aymerich, Costa, Monfort, & Hugas, 2002). Ponce, Pla, Sendra, Guamis, and Mor-Mur (1998) used the combined effect of high hydrostatic pressure with nisin in liquid whole egg for destruction of *Listeria innocua* and *E. coli. Pseudomonas* has also been reported to be inhibited with the combined effect of high hydrostatic pressure with lactoferrin, lactoferricin and nisin (Masschalck, Houdt, & Michiels, 2001). Yuste, Pla, Capellas, and Mor-Mur (2002) observed significant decreases in mesophile and psycrotroph bacteria counts of mechanically recovered poultry meat by the combined treatment of high pressure (450 MPa) and nisin at 20 °C. Organic acid applications especially lactic and acetic acids became another practice, used to decontaminate meat and meat products for years. Netten, Mossel, and Veld (1995) decontaminated pork carcasses artificially contaminated with *S. typhimurium* with 2% and 5% lactic acid for 30–120 s and determined 1–2 log reduction. Similarly, Stivarius, Pohlman, McElyea, and Waldroup (2002) used the combined effect of hot water and 5% lactic acid on beef trimmings prior to grinding and observed significant reductions of *S. typhimurium* and mesophilic aerobic bacteria (MAB). According to Smulders and Greer (1998), decontamination of subprimal and retail meat cuts with lactic acid is very efficacious. These researchers claimed 1.5 log reduction in surface contaminants (especially *Yersinia enterocolitica*) of carcasses. The acidity maintained with the yogurt dip of chicken carcasses at the critical point between spray washing and air chilling (marked in Fig. 1) and then nisin application, could be synergistic in eliminating *Salmonella* and inhibiting MAB. This study was designed in order to observe this synergism and the effect of yogurt dip, nisin and coating alone, and combined applications on microbiological and sensory quality of poultry meat at a refrigeration temperature of (+4 °C).

2. Materials and methods

Yogurt and chickens were purchased from a local market. The coating material was prepared according to the recipe of Daniels (1973) by homogeneously mixing 46.6% vegetable oil, 1.8% beeswax and 51.6% water with heat application until all wax melted. Nisin was obtained from SIGMA Chemical Co. (St. Louis, MO). 10 ml aliquot was transferred from enriched *Salmonella* stock suspension (which contains 7×10^4 cfu/ml *S. typhimurium*) into 15 l tap water and mixed homogeneously. Followed by evisceration & spray washing (Fig. 1), carcasses were dipped in this water (containing 7×10^5 cfu/15 l *S. typhimurium*) and incubated for 20 min, immersed to allow *Salmonella* penetration to the carcasses.

Contaminated carcasses were randomly divided into 10 groups. First group carcasses as untreated were kept as control. Each carcass of the second group was dipped into 3 l tap water with a pH of 5.2 containing 0.04 g nisin for 20 min. The third group of carcasses was treated the same with nisin for 20 min, and in this case the coating was used additionally. Carcasses of the fourth group were only coated while the fifth group of carcasses were immersed and incubated in yogurt for 3 h. The sixth group of carcasses were immersed and incubated in yogurt for 5 h. The seventh group of carcasses were additionally treated with nisin for 20 min followed by the immersion in yogurt for 3 h. The carcasses in the eighth group were treated with nisin for 20 min followed by the immersion in yogurt for 3 h and coated. The ninth group was immersed in yogurt for 5 h and subsequently treated with nisin for 20 min while the last group of carcasses was immersed in yogurt for 5 h,

Table 1
The codes of application groups

C	Control
N	Nisin treated group
Co	Coated
3Y	The group which was dipped in yogurt for 3 h
5Y	The group which was dipped in yogurt for 5 h
NCo	Nisin treated and additionally coated
3YN	Yogurt treated for 3 h then nisin treated
5YN	Yogurt treated for 5 h and then nisin treated
3YNCo	Yogurt treated for 3 h, nisin treated then coated
5YNCo	Yogurt treated for 5 h, and nisin treated then coated

treated with nisin and coating. All samples were kept in duplicate sterile plastic bags at 4 °C. The codes are given in Table 1.

0.5 g from the breast, 0.5 g from tail and 0.5 g from abdominal cavity of each carcass in each group were ground and mixed homogeneously by sanitized lancet and tweezer. One gram from these ground meats resembling each group were assayed for microbial criteria. Serial dilutions in peptone water were prepared from the ground and homogenized samples. Enumeration was carried out by using PCA (Oxoid) for MAB, MacConkey Agar (Oxoid) for *Pseudomonas* and Salmonella-Shigella Agar (Oxoid) for *Salmonella*. Spread plating for *Pseudomonas* and pour plating for MAB and *Salmonella* enumerations at 37 °C for 24–48 h as in duplicates were carried out for each sample. The pH of the samples was measured after homogenization, using a digital pH meter (Heidolph-Schott, Maintz). Microbial enumeration and pH determinations were made in duplicate 24 h after applications, and then continued at 24 h' intervals.

Lactic acid levels were determined by high-performance liquid chromatography (HPLC). LKB-Bromma model 2150 HPLC equipment was used, equipped with a differential refractometer (KNAUER) and Shimadzu C-R-4-Chromatopack monitor. The column used was a Phenomenex Rezex Cal monosaccharide column, with a size of 300×7.8 μm. The attenuation parameter was set to 2, speed = 2, flow rate was 0.45 and the oven temperature was kept as 55 °C throughout the experiment.

Considering all the experimental trials, since nisin and coating applications did not affect meat sensory quality, only yogurt treated groups, 5Y and 3Y, were included in a taste panel by fifteen panelists having a background of sensory quality training. Tenderness, texture, appearance, color, taste, odor and juiciness criteria of the application groups were evaluated by giving points between 1 (the worst) and 10 (the best).

Multivariate statistical analysis (Richard & Whichern, 1987) was applied to determine variance between the sole and combined effects of nisin, yogurt and coating applications. The degrees of difference between applications were determined by Duncan's multiple choice test (Scheffe, 1970) while Fcalc test was used to evaluate the taste panel results since there were only two groups to be compared with the control.

3. Results and discussion

According to the experimental results; "5 h of yogurt treatment followed by nisin and coating" (5YNCo) significantly inhibited MAB growth during the refrigerated storage period ($p < 0.01$). MAB count for 5YNCo was kept around 5.86 log cfu at the end of the 6th day whereas the control had reached 6.01 log cfu at the end of the 4th day which is within the limits of consumption risk. 5YNCo had only reached 5.86 log cfu whereas control indicated 7.97 log cfu at the end of 6th day. The 2.11 log reduction of MAB due to the effect of 5YNCo was also consistent with 1.8 and 1.5 log reductions of MAB indicated by Nettles and Siragusa (1994) and Smulders and Greer (1998) respectively. 2.11 log reduction of MAB due to the "yogurt acidity & nisin" in this study was even compatible with the 2.3 log reduction due to the "hydrostatic pressure (500 MPa)" applied by Yuste, Mor-Mur, Capellas, and Pla (1999). "5 h yogurt treatment followed by nisin" (5YN), "3 h yogurt treatment followed by nisin and coating" (3YNCo) and "5 h yogurt treatment" (5Y) applications respectively, were found to be less effective compared to the 5YNCo on inhibiting MAB growth ($p < 0.01$). Coating alone was the least effective on MAB growth and as comparable to the inhibition of nisin used alone (Fig. 2 and Table 2).

5YN application showed the most significant inactivation of *Salmonella* with 1.97 log reduction while the control had the highest *Salmonella* count ($p < 0.01$) on the 6th day of incubation at 4 °C. 5Y and 5YNCo followed by 5YN, appeared significantly inhibitory for *Salmonella*. In comparison, statistical analysis indicated significant synergism of nisin and yogurt from the point of inhibition of *Salmonella* ($p < 0.01$) (Fig. 3 and Table 2). 1.97 log reduction of *S. typhimurium* due to the synergistic effect of "yogurt acidity and nisin" was also supported by the 1.5 and 1–2 log reductions of *S. typhimurium* reported by Gill and Holley (2000) and Netten et al. (1995) respectively. Similar to the control, nisin (N) and "nisin then coating" (NCo) applications appeared as the least effective treatment groups since nisin was only inhibitory for Gram positive flora and *Salmonella* facultatively anaerobic bacterium.

Pseudomonas spp. which are members of the microflora of the product at refrigerated storage were found to be significantly inhibited by coating (Co) and the combined application of coating with nisin (NCo) ($p < 0.01$). 5YN also significantly inhibited *Pseudomonas*, followed by N and NCo applications. The 0.51 log reduction resulting from 5YN application was also consistent with 1 log reduction due to the synergistic

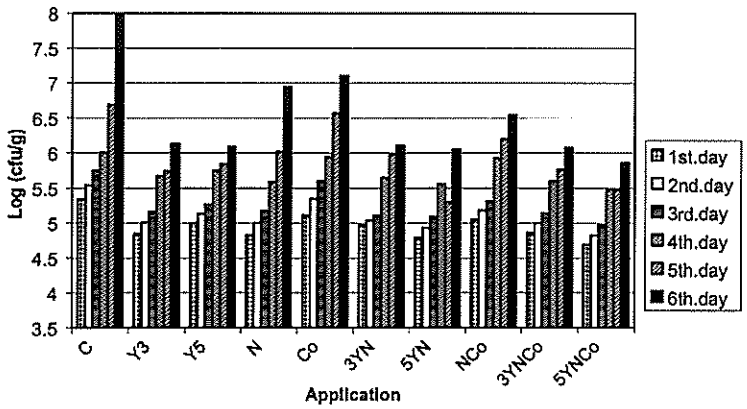


Fig. 2. MAB counts of control and application groups.

Table 2
Variation among the effects of nisin, coating and yogurt treated application groups on quality criteria of chicken meat

Criteria	Application									
	Co	NCo	5YN	3YN	5Y	3Y	5YNCo	3YNCo	C	N
Pseudomonas	a*	ba	ba	b	bc	c	d	de	e	g
Salmonella	c	c	a	ba	ba	ba	ba	b	d	c
MAB	bc	b	ba	ba	ba	ba	a	ba	c	bc
pH	j	g	b	d	c	f	a**	e	g	h
Total lactic acidity (%)	c	d	ba	bc	a***	ab	bc	bc	e	c

* The highest inhibition, lowest count ($p < 0.01$) (a → g indicates increase in microbial count).
** The lowest pH level ($p < 0.01$) (a → j indicates increase at pH level).
*** The highest total lactic acidity level (%) ($p < 0.01$) (a → e indicates decrease in total lactic acid level (%)).

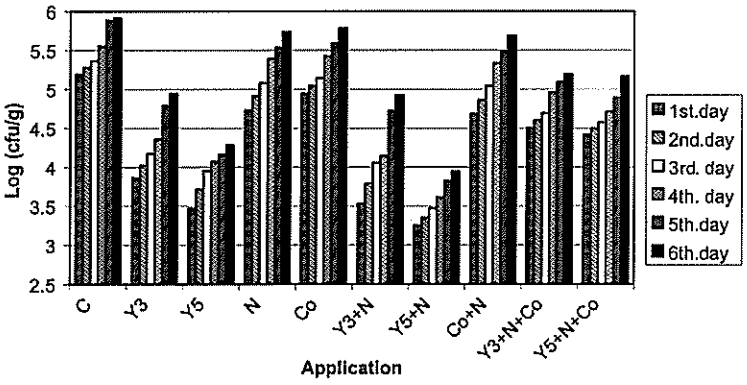


Fig. 3. *S. typhimurium* counts of control and application groups.

effect of lactoferrin, lactoferricin, high hydrostatic pressure and nisin, observed by Masschalck et al. (2001). The significant reduction observed in coating in this study was possibly due to the creation of an anaerobic environment. Other applications were found to be less inhibitory while nisin and control were least effective ($p < 0.01$) (Fig. 4 and Table 2).

According to the taste panel, there was no significant difference among three of the sets (5Y, 3Y and control) in taste, odor, color and juiciness criteria, whereas tenderness, texture and appearance were effected positively in 5Y and 3Y applications respectively, compared with the control ($p < 0.01$).

5YNCo indicated the lowest pH values in all periods of shelf life orderly followed by 5YN, 5Y, 3YN, 3YNCo and 3Y, while the highest pH levels were observed in the control ($p < 0.01$) as clearly indicated in Fig. 5 and Table 2. The lower pH levels of these groups were also confirmed by their higher total lactic acidity contents (Fig. 6 and Table 2).

When different applications are compared, it is obvious that 5YNCo and 5YN show better inhibition toward

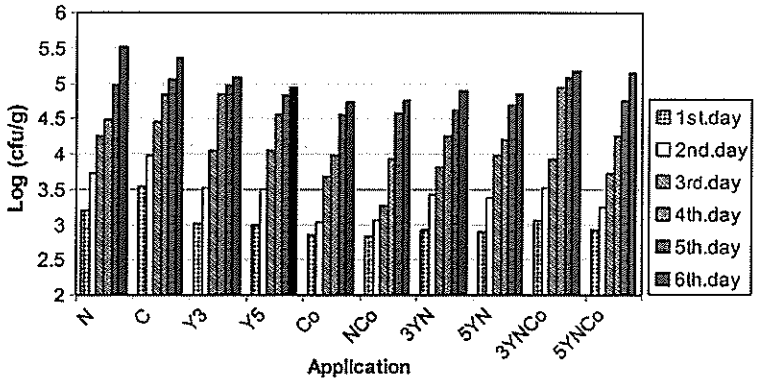


Fig. 4. *Pseudomonas* spp. counts of control and application groups.

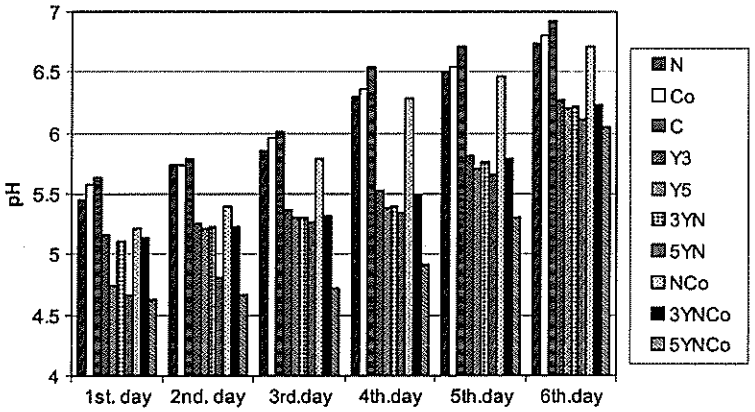


Fig. 5. pH levels of control and application groups.

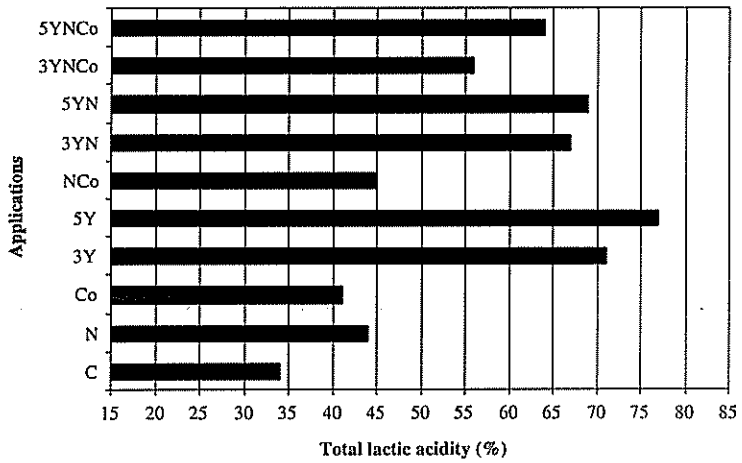


Fig. 6. Total lactic acidity (%) levels of control and application groups.

MAB and *S. typhimurium* while 5YN is also inhibitory to *Pseudomonas* spp. This can also be seen from the lower pH levels of these groups (Fig. 5). The lower *Salmonella* and *Pseudomonas* counts of 5YN in comparison with 5Y, shows a synergism against these microorganisms while coating provides anaerobic conditions inhibiting the growth of *Pseudomonas* spp. (Fig. 4 and Table 2). Inability of any growth inhibition by nisin alone of *Pseudomonas* spp. was expected due to its ability to effect only the Gram positive population.

In conclusion; although nisin alone had no significant effect on Gram negative bacteria, combination of nisin

with a yogurt dip of chicken carcasses, with or without coating, can be successfully applied to retard the growth of both Gram positive and Gram negative *S. typhimurium* and *Pseudomonas* spp. spoilage flora which may contaminate carcasses during evisceration. Not only 5YN and 5YNCo applications are preventative for such contamination in the critical point between spray washing and air chilling, but also yogurt dip for 3 or 5 h. can be strongly advised for home preparations as improving microbial and sensorial quality of chicken meat at refrigeration temperatures.

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Professor Chris Smith

Chairman of the Local Organising Committee

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Useful Links

- University of Chester Homepage
- Learning and Enterprise Centre

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- Location
- Campus Map