Global Veterinaria 9 (3): 284-289, 2012
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2012.9.3.1827

Study on the Antimicrobial Effect of Nanosilver Tray Packaging of Minced Beef at Refrigerator Temperature

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Abstract: Fresh meat is a highly perishable commodity. Under normal aerobic packaging conditions, the shelf life of refrigerated meat is limited by the growth of bacteria. Silver nanoparticles have recently attracted increasing interest because of their antimicrobial activities in food packaging. This study was conducted to evaluate the antimicrobial effect of nanosilver packaging at refrigerator temperature (3±1°C) in minced meat (beef). Two thickness of minced meat (1.5 and 1cm) were packed in two types of packaging; (1) tray that was fortified by nanosilver particles and (2) common packaging. Samples were examined for total bacterial, *E.coli* and *S.aureous* count on day 1, 3, 7, 10 and 14. The microbial counts indicated that nanosilver packaging reduced the microbial growth of minced meat. Shelf life of 2 days in common food packaging was extended to 7 days with similar acceptability scores in nano-silver packaging samples. The antimicrobial effect of the nanosilver tray on thin minced meat packaging was higher than thick minced meat packaging. We believe that nanosilver tray improved the quality and shelf life of minced meat at refrigerator temperature compared with common packaging.

Key words: Nano-silver packaging · Tray · Antimicrobial · Shelf-life · E.coli · S.aureous

INTRODUCTION

Fresh meat is a highly perishable commodity because of its biological composition [1, 2]. At the time of slaughter, meat obtained from a normal, healthy animal may be regarded as essentially bacteria-free nevertheless, contamination of the meat may originate from the animal, hands of personnel and equipment [2]. Meat spoilage mainly results from microbial growth [3, 4]. As shelf life is one of the problems of packaged meat, research to improve the methods of transporting, packaging and storing food products continues [5, 6]. Under normal aerobic packaging conditions, the shelf life of refrigerated meat is limited by the growth and activities of aerobic bacteria [7, 8]. In the meat industry, the possibility of increasing the shelf life of meat by additional control methods is one of the main objectives of research [9]. Modified Atmosphere Packaging (MAP) is one of them, but MAP is expensive and requires operator training and equipment [7]. In the last decade, nanotechnology had impressive effects on various areas of human life, particularly in the food sectors [10, 11]. In the food sector, nanotechnology can be used in the processing, packaging and storage of food products [5, 10, 12]. Silver nanoparticles (Ag-NPs) have recently attracted increasing interest [10] because among heavy metal nanoparticles, Ag-NPs are known to possess inhibitory and bactericidal effects against both gram-positive and gram-negative bacteria [9, 10, 13, 14, 15]. Antimicrobial Ag-NPs can be used in packaging and storage containers to increase the shelf life of food products when used as nano films or nano trays [11, 12, 16]. Emamifar used nano-silver film packaging to extending the shelflife of orange juice, her study indicated that containing nanosilver packaging; increased shelf life of fresh juice although part of its sensory attributes were lost [16]. This study was primarily designed to evaluate the antimicrobial effect of nanosilver packaging in minced beef during storage at refrigerator temperature.

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METHOD AND MATERIALS

Packaging: In this study used from 4 types of minced meat (beef) packaging, beef meat obtained from a single carcass. Two nano-silver tray packaging and two common packaging (control), nano-silver packaging and common packaging were prepared in two thicknesses of minced meat (10-15 mm). The minced meat packed in nano-silver tray labeled as *a** and *b** (*a**: thin; *b**: thick) and minced meat in common packaging labeled as *c** and *d** (*c**: thin; *d**: thick). All packages covered by common food films. In control groups (*c** and *d**) used from common trays (polyvinylchloride-polyethylene laminate trays (160 x 125 x 35 mm)), Nano-silver trays was made from same P.V.C. packaging, that coated with 5% nano-silver particles (by inkjet-printed method), the size of nano-silver particles was 40-50 nm.

Storage: All samples were stored for up to 14 days at 3±1°C in a chilling room that was air ventilated to prevent rapid spoilage of the minced meat. The samples were investigated at different times of storage, namely 1, 3, 7, 10 and 14 days.

Preparation of Samples: The entire content of the trays was mixed thoroughly and then 25g portion taken from mixed minced meat and this portion was homogenized with 225 ml sterilized peptone water (PW) for 1 min in a sterilized bag using a stomacher blender (Lab Blender 400, Seward, Worthing, UK). When homogenized, serial dilutions and appropriate dilutions of the samples were spread on plates (agar culture) or inoculated into tubes (broth culture).

Microbial Examination: Total mesophilic bacteria (TC) were counted using plate count agar (PCA; Liofilchem serial: 610040). The plates were incubated at 30°C for 72 h [17, 18]. For *E.coli* enumeration, MPN (3 tubes) procedure is used for the analysis of samples. In the first step, tubes that contain LST broth are inoculated according MPN procedure. Tubes are incubated at 35±5°C 24 and 48±2 hr, after inoculation. Tubes are examined for evidence of gas production in the Durham tube at the end of 24 hr. Negative tubes are re-incubated for 24 hr and examined for gas production again. In the second step, from all LST tubes that positive gas production within 48±2 hr, subcultured into BGB broth by means of 3-mm loop. Then all BGB broth incubated at 35±0.5°C and 45.5±0.5°C for 48 ± 2 hr. Gas production in these time and temperatures were considered confirmation of coliform and fecal coliform presence respectively. In third step, from all LST broth tubes that were positive in gas production within 48±2 hr, subcultured into EC broth tubes and all tubes incubated at 45.5±0.5°C for 48±2 hr. All EC broth that exhibiting gas production within 48±2 hr, subcultured in EMB agar plates and peptone water broth; then P.W. tubes and EMB plates incubated in 35°C for 24 hr. Examine plates for green metallic sheen which were indicative of *E.coli* and tubes that indol positive; [19]. *S.aureus* was counted according to international standard procedures Appropriate dilutions were spread on Baird–Parker agar plates (MERCK; 1.05406.0500). The plates were incubated at 37°C for 48 h. Mannitol and coagulase tests were performed on typical colonies [20].

Statistical Analysis: Data were analyzed for normality and homogeneity of variance. The effects of nanosilver packaging on the microbial properties of minced beef were evaluated by paired samples Student's *t*-test. Differences related to the thickness of the minced meat (i.e., *a** and *b**) were evaluated by Student's *t*-test. To determine the difference between the nanosilver packaging and control groups, two-way analysis of variance (ANOVA) was used. Statistical analyses were performed using SPSS version 16.0.0.247 software (State-packets, statistical analysis software, SPSS Inc., Chicago, IL) and the level of significance (*P* value) was set at 0.05. the study were replicate two times.

RESULTS

According to the result, the control group samples (*c** and *d**). had higher microbial counts than minced meat that packed in nano-silver packaging samples (*a** and *b**).

The total colony counts in both thickness of minced meat in control and nano-silver packaging on PCA during 14 days of study is shown in Fig. 1. Analysis of the data indicated that the total count significantly increased ($P<0.05$) in all packaging during storage.

Based on the result of our study, a significant difference was observed between the initial day and day 14 in *E.coli* counts ($P<0.05$) in packaging (*a**, *b**, *c** and *d**). *E.coli* counts in minced beef in thin and thick nano-silver and control packaging is shown in Fig. 2.

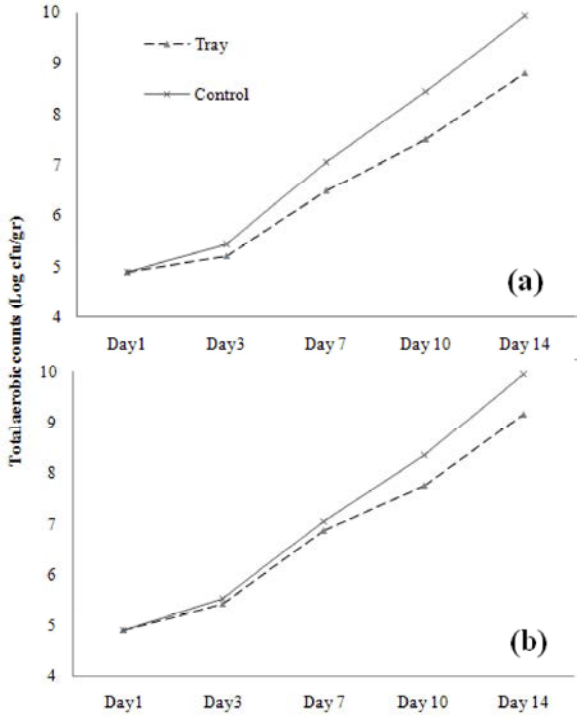

Fig. 1: Total aerobic bacterial counts of minced beef packed in thin (a) and thick (b) packaging (Log cfug), n=40

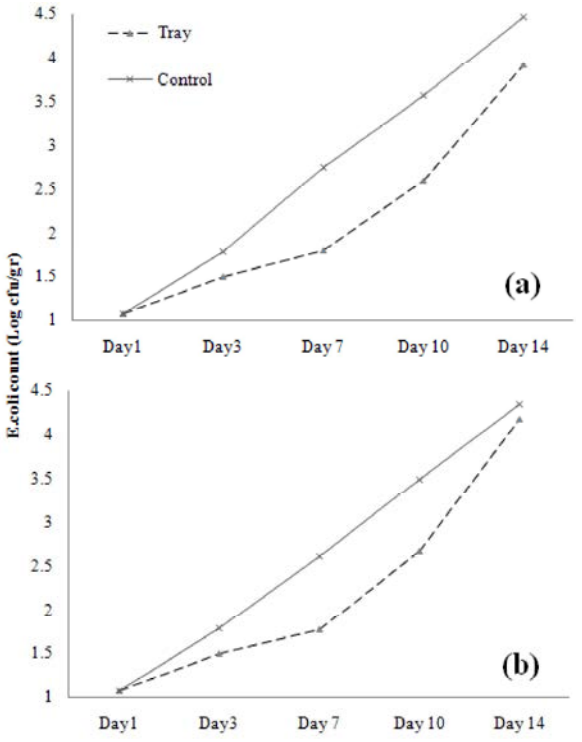

Fig. 2: E.coli counts of minced beef packed in thin (a) and thick (b) packaging (Log cfug), n=40

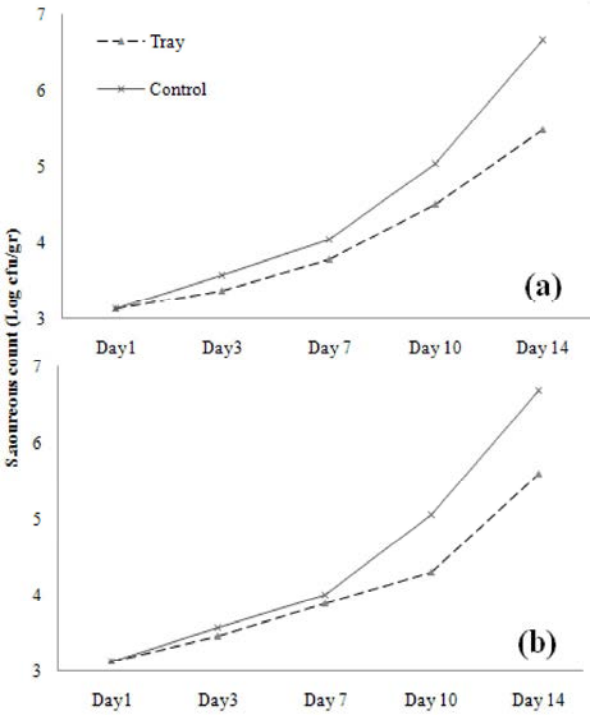

Fig. 3: S.aureus counts of minced beef packed in thin (a) and thick (b) packaging (Log cfug), n=40

Fig. 3 show *S.aureus* counts in minced meat. The *S.aureus* counts increased significantly (P<0.05) in all packaging after 14 days of study.

DISCUSSION

According to our results, significant differences were observed between nano-silver packaging and the control groups (P<0.05). According to the national standard of Iran, the maximum acceptable value of total microbial count for minced meat was 5.10^6 cfu/g [21], this study indicated that bacterial count in packaging *a** was under this level on day 7 and total microbial count in common food packaging samples on day 7 was higher than the standard level. Our results demonstrated the inhibitory effect of nano-silver packaging on microbial growth, particularly in packaging *a**. The inhibitory effect of nanosilver on microbial growth has been previously discussed. Cho [13] investigated the antimicrobial activity of Ag-NPs. He suggested that Ag-NPs, but not platinum and gold nanoparticles, have antimicrobial activity. Rai [10] described Ag-NPs as a new generation of antimicrobials. Shahverdi [22] investigated the antibacterial activity of Ag-NPs against gram-positive and

gram-negative bacteria with an emphasis on their antibacterial effects. Lkhagvajan [23] investigated the antimicrobial activity of colloidal Ag-NPs and found that Ag-NPs inhibit the growth and multiplication of all tested microorganisms.

The count increased significantly (P<0.05) in all samples after 14 days and significant differences were observed between the thin nano-silver packaging and thin control groups on day 14. In the thick minced meat packaging, no significant differences were observed during study. So although nano-silver packaging decreased the *E.coli* count of minced meat compared control group samples (Fig.2) but had no significant inhibitory effects on *E.coli* growth in thick minced meat packaging. According to the national standard of Iran, the maximum acceptable value of *E.coli* for minced meat was 5.10^2 cfu/g [21, 24]. and nano silver packaging was under this level on day 10 but *E.coli* count in common food packaging on day 7 was higher than the standard level.

The low *E.coli* counts in the nanosilver packaging group than in the control group were consistent with the results of studies by Sondi [25] and Cho [13]. Shahverdi [22] investigated the antimicrobial effects of Ag-NPs against *E.coli*. Jun [26], Li [27] and Raffi [28] reported the bactericidal effects of Ag-NPs against *E.coli*. Li [15] found that Ag-NPs at certain concentrations could completely inhibit the growth of *E. coli* cells in liquid Mueller–Hinton medium. Jain [29], Humberto [30] and Lkhagvajan [23] individually investigated the effects of Ag-NPs on *E.coli* growth and demonstrated that Ag-NPs inhibit *E.coli* growth. In our study; The nanosilver packaging had better inhibitory effects against *E. coli* growth in the thin sectioned mince meat samples than in the thick sections of minced meat.

According to our results, significant difference was observed between the thin nano-silver packaging and control groups on day 7. The differences between the thick minced meat nano-silver packaging and control groups observed on day 14. The *S.aureus* counts at 14 days in the nano-silver packaging were lower than those in common food packaging. According national standard of Iran, the maximum acceptable value of *S.aureus* for minced meat was 5.10^3 cfu/g [21], this study indicated that *S.aureus* count in packaging *a** was under this level on day 7 and *S.aureus* counts in common food packaging samples (*c** and *d**) on day 7 was higher than standard level. In this study, the growth of *S.aureus* and *E.coli* in the nano-silver packaging was slow. These results were consistent with those of previous studies by Cho [13]. Shahverdi [22] investigated the antibacterial activity of Ag-NPs against *S.aureus*. Lkhagvajan [23] found Ag-NPs to be effective against *S.aureus* growth; these results were consistent with those of Panacek [31] and Jun Sung [26].

Samples packed in *a** and *b** were acceptable after day 7 based on TC (under 5×10^6 cfu/g), *E. coli* (under 5×10^2 cfu/g) and *S.aureus* (under 5×10^3 cfu/g) counts [21, 24]. An indistinct or slight off odour could be detectable at this point, but not as unacceptable odours. However, samples packed in common food packaging (*c*, d**) had spoiled at 2 days of storage. This is agreed with [1], he noted that under an optimum storage condition, the shelf life of minced beef packed in common food film packaging is 2 days after preparation and packaging. At day 10, all samples were a dark brown/grey and had very strong putrid odour, particularly the samples in the common food film packaging (*c*, d**).

The microbial profile (load) of minced beef stored in common film packaging was significantly higher than that stored in nano-silver packaging. The short shelf life of the common packaging samples was a result of increases in microbial counts [3, 4].

The differences in micro flora after storage between the samples in nano-silver packaging and control groups were because of the bacteriostatic effect of nano-silver present in the trays. Based on the results of this study, the storage period (P<0.01) and nano-silver packaging (P<0.01) had significant effects on the amount of microbial load of the minced beef. However, the microbial results of the thin minced meat packaging were lesser than those of the thick minced meat packaging. Statistical analysis of data indicated that the thickness of minced meat had no significant effect on these parameters. Nevertheless, we believe that if the thickness of minced meat had been lesser or the concentration of nano-silver (in tray) was higher than what was used, the results would have been significant. This is agree with previous research that higher concentration of nano-silver had stronger antimicrobial effect [10, 14]. So we believe the nano-silver packaging could be useful for small mass of minced meat such as hamburgers.

Nano-silver packaging preserved the microbial properties of minced beef better than common food packaging and improved its microbiological shelf life. Nano-silver packaging (tray) with a higher concentration of nano-silver combined with a lower thickness may lead to increased shelf life compared with only higher concentration of nano-silver or a lower thickness.

Based on the results of this study, nano-silver packaging could be one of the preferred choices of packaging for preserving the overall quality of minced beef and increase its shelf life. The following conclusions were drawn from this study:

Nano-silver packaging reduce microbial growth and compared with plain packaging, antimicrobial nano-silver packaging (a^* and b^*) as an alternative non-thermal technology can increase the shelf life of minced beef up to 7 days (from 2 days).

These results indicate that applying nano-silver packaging could increase the shelf life of minced meat.

If nano-silver packaging was combined with additional effective hurdle effects, such as vacuum, modified atmosphere packaging (MAP) or freezing, the spoilage time would be significantly increased compared with solely pure nano-silver packaging.

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