COMBINED EFFECT OF γ-IRRADIATION AND ANTIMICROBIAL FILMS ON THE SHELF LIFE OF REFRIGERATED PORK SAUSAGE MEAT

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Abstract – The objective of this study was to evaluate the effect of biopolymeric antimicrobial films, combined with low dose γ-irradiation (0.5 and 1.5kGy) on the microbiological quality of fresh pork sausages meat. The antimicrobial formulations (lactic acid + Biosecur® + oregano essential oil) were incorporated into the biopolymeric matrix, homogenized and films were made by casting. Pork sausages were prepared by first mixing with a binding agent and sandwiched between two antimicrobial films. The samples were irradiated using a UC-15A irradiator equipped with a ^{60}Cobalt source. The γ-irradiation treatment alone was able to reduce the initial psychrotrophic and mesophilic bacteria by more than 2 and 1 log CFU/g, respectively. The initial lactobacillus population was also under the detection limit (100 CFU/g). Combination of antimicrobial films with γ-irradiation treatment reduced both the psychrotrophic and mesophilic bacteria by more than 3 log CFU/g at day 13 and increased the shelf life of the pork sausages from 7 to 15 days. This study demonstrated that use of antimicrobial films with low dose of γ-irradiation can be applied to extend the shelf life of fresh pork sausages meat.

Key Words – Antimicrobial films; γ-irradiation; pork sausages.

I. INTRODUCTION

Antimicrobial packaging is gaining interest from researchers and industries due to its potential to provide quality and safety benefits. The reason for incorporating antimicrobial agents into the packaging is to prevent surface growth of micro-organisms in foods where a large portion of spoilage and contamination occurs [1]. Chitosan, a natural linear polysaccharide consisting of 1,4-linked 2- amino-deoxy-β-D-glucan, is a partially deacetylated derivative of chitin, the second most abundant natural polysaccharide after cellulose. Chitosan was found to be non-toxic, biodegradable, biofunctional, biocompatible and has strong antimicrobial and antifungal activities [2]. Antimicrobial agents such as, organic acids (e.g. lactic, propionic, acetic acids, etc.), organic citrus extract (Biosecur®) and spice extracts (e.g. essential oils) can be added into films to reduce bacterial growth on meat products [3]. Gamma irradiation is a well-known method to improve the safety and extend the shelf life of food products by protecting it against pathogenic bacteria and spoilage microorganism [4]. The objective of this study was to evaluate the effect of antimicrobial films combined with low dose γ-irradiation on the microbiological quality of pork sausages during storage at 4°C.

II. MATERIALS AND METHODS

Preparation of antimicrobial film

A 2% chitosan (Heppe-medical chitosan, GmbH, degree of deacetylation 82.6-87.5%) was dissolved in 1% aqueous acetic acid solution. The suspension was stirred for 24 h with a magnetic stirrer. Nanocrystalline cellulose (NCC, generously provided by FPInnovations) was incorporated into the chitosan suspension (NCC content was 5% by wt. in the dry film) and homogenized. The antimicrobial formulation (AF) was incorporated into the chitosan/NCC suspension and homogenized at 23,000 rpm for 2

min by an IKA T25 digital Ultra-Turrax disperser (IKA Works Inc., Wilmington, NC). The AF contained lactic acid + Biosecur® +oregano essential oil + Tween® 80. The concentration of AF in the films forming suspension were 3% w/v lactic acid, 0.3% w/v Biosecur®, 0.03% w/v oregano essential oil and 0.06% w/v Tween® 80. Films were made by casting and allowed to dry. After drying the films were treated with 0.5 M NaOH and washed several time to render the films insoluble. Two types of films with AF and without AF (control) were prepared.

Preparation of sausage meat

Sausage meat was prepared by mixing ground pork with a binding agent (BSA Food Ingredients, St-Leonard, Qc, Canada). The binding agent contained toasted wheat crumbs, salt, sugar, spices, silicon dioxide, polysorbate 80 and canola oil. Cold water was added to the preparation and it was mixed for 5 min. The meat, binding agent and water proportion that were used are 69.9%, 8.15% and 22.05%, respectively. Approximately 10 g of sausage meat was sandwiched between two films and were kept in a sterile bag at 4 °C until analysis. Six different formulations of sausages meat were prepared (control, AF, Control+0.5 kGy, AF+0.5 kGy, Control+1.5 kGy, and AF+1.5 kGy).

Irradiation

The sandwiched sausage meat samples were irradiated with 2 different radiation doses (0.5 and 1.5 kGy). A UC-15A irradiator (Nordion Inc., Kanata, Ontario, Canada) equipped with a ^{60}Cobalt source was used to deliver radiation at a mean rate of 16.2 kGy h^{-1}. The radiation treatment was carried out at the Canadian Irradiation Centre (Laval, Quebec, Canada) at room temperature (20°C).

Microbial analysis

Sausage meat samples were analyzed for microbial growth after 1, 5, 9, 13 and 17 days of storage. On each day of analysis, 3 samples from each treatment were diluted 10 fold with peptone water (0.1%; BD, Sparks, MD, USA) using a mechanical homogenizer for 1 min at 200 rpm in a sterile filter sample bag (Whirl-Pak; Nasco, Fort Atkinson, WI, USA). Dilutions were plated onto tryptic soy agar (TSA; BD) for the enumeration of psychrotrophic and mesophilic bacteria and were incubated at 15°C and 37°C, respectively, for 48 h. For the enumeration of *Lactobacillus* species present in the sausage, dilutions were plated onto MRS agar (BD) and incubated under anaerobic conditions at 37 °C for 48 h. Following incubation, the colony forming units were counted using a magnifier. Detection limit for all microbial analysis was 100 colonies forming unit (CFU) per g.

Statistical analysis

All bacterial counts were log10 transformed prior to statistical analysis. The mean of three samples per day of analysis were used for each group. An analysis of variance (ANOVA) and multiple comparison tests of Duncan's were used to compare all the results. Differences between means were considered significant when the confidence interval was smaller than 5% ($P \leq 0.05$). The analysis was performed by the PASW Statistics 18 software (SPSS Inc., Chicago, IL, USA).

III. RESULTS AND DISCUSSION

Total psychrotrophic bacteria

Microbiological analyses of psychrotrophic bacteria in fresh pork sausages are shown in Fig. 1.

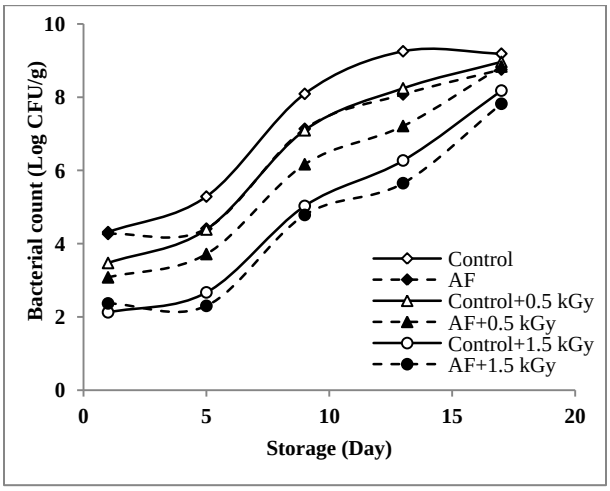

Figure 1. Population of psychrotrophic bacteria in fresh pork sausage meat during storage at 4° C.

Films containing antimicrobial formulation (AF) did not reduce the initial bacterial population. However, AF did manage to reduce the bacterial count during storage. The bacterial count was reduced by 0.87, 0.95, 1.18 and 0.44 log CFU/g

during storage at 3, 7, 13 and 17 days. Gamma irradiation at doses of 0.5 and 1.5 kGy reduced the bacterial count throughout the storage compared to the control (without irradiation). Samples irradiated at 1.5 kGy reduced the psychrotrophic bacteria count by 2.98 log CFU/g compared to the control, at 13 day. Combination of antimicrobial films with 1.5 kGy irradiation (AF+1.5 kGy) further reduced the bacterial count 0.62 log CFU/g.

Total mesophilic bacteria

Microbiological analyses of mesophilic bacteria in fresh pork sausages are shown in Fig. 2.

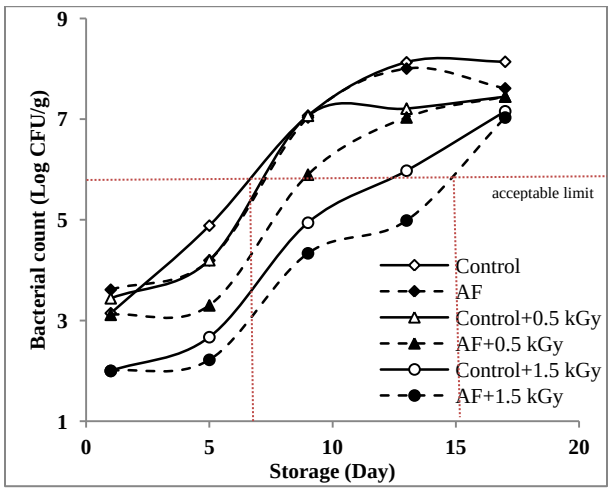

Figure 2. Population of mesophilic bacteria in fresh pork sausage meat during storage at 4° C.

Results demonstrate that AF was not able to reduce the bacterial population during storage. Irradiation dose of 1.5 kGy was found to be very effective on the reduction of mesophilic bacteria. Bacterial count was beyond detection limit at day 1 for the control+1.5 kGy and AF+1.5 kGy formulations. At day 13, the total mesophilic bacterial count was found to be 5.98 log CFU/g, which is almost 2.15 log CFU/g lower than the control samples. A further 1 log CFU/g ($P \leq 0.05$) reduction was achieved with the AF+1.5 kGy formulation.

Lactic acid bacteria (LAB)

Lactobacillus species are generally considered to be responsible for the degradation of packaged meat products (5). Results of microbiological analyses of LAB in fresh pork sausages are shown in Fig. 3. Initial (Day 1) LAB contamination in the sausages was low. But during storage (1 to 17 days) the LAB grew and after 17 days the count was 7.98 log CFU/g. The AF (without any irradiation treatment) was not found to be effective against the growth of LAB. However, γ-irradiation treatment (1.5 kGy) was able prevent the growth of LAB and after 17 day the count was 6.39. Maximum reduction of LAB occurred at day 13. The formulation control+1.5 kGy reduced the count by almost 3 log CFU/g.

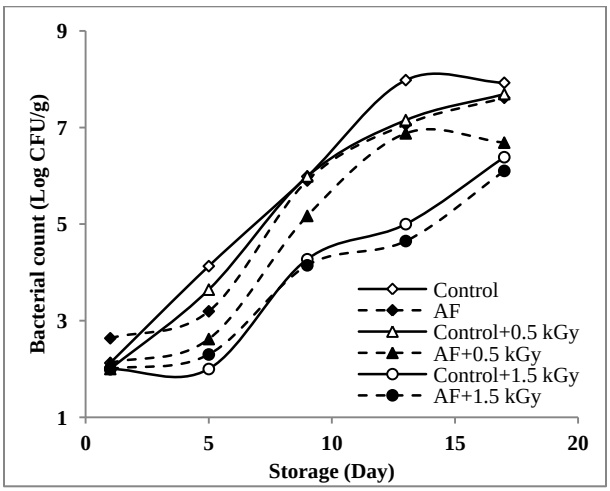

Figure 3. Population of LAB in fresh pork sausage meat during storage at 4° C.

IV. CONCLUSION

This study showed that by adopting innovative technology such as, γ-irradiation in combination with natural antimicrobial biopolymeric films, it is possible to extend the shelf life of fresh pork sausages. Low dose γ-irradiation (1.5 kGy) alone reduced bacterial growth and the growth was further reduced by the combined treatment. So, use of antimicrobial films along with γ-irradiation can be considered as suitable technique to improve the safety of packaged meat products.

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