GROWTH INHIBITION OF MICRO-ORGANISMS CAUSED BY SPOILERS AND PATHOGENS FOUND IN MEAT PRODUCTS USING CHICKEN IMMUNOGLOBULIN Y

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Abstract— **This report divided into two parts; the first part focuses on the production of hyperimmunized eggs by immunizing chickens with 12 different bacteria. The eggs with high level of immunoglobulin yolk (IgY) against bacteria were processed for a powder form of IgY cocktails, called SpiceGuard™. The second part is to test the antimicrobial activity of the SpiceGuard™ in a meat sample. Different concentrations of the SpiceGuard™ (0, 0.1%, 0.2%, 0.5%) were mixed with a pork meat to make a pork patties and sausages. The meat then stuffed into natural hog casing and cooked in a smokehouse and then stored. The Results showed that using 0.5% SpiceGuard™ can reduce the growth of aerobic bacteria in pork patties and sausages stored at 10°C for 7 days by at least one log.sub.10.**

Index Terms— ELISA, IgY, bacteria, pork patties, sausages

I. INTRODUCTION

The demand of fresh and highly nutritious food free from synthetic preservatives is vastly increasing due to the growing health awareness as well as changes in consumers' lifestyle. Ready-to-eat food product is getting popular as a result of this high demand by consumers for convenient food products that require least preparation procedure and time. This type of food relies mainly on a mild heat treatment and refrigerated storage conditions. These processes raise serious questions about the safety of the food from the microbiological point of view (Song, Kim, Cho, & Sunwoo, 2009). Foodborne transmission of disease in the United States has been estimated to cause 13.6 million cases of illness and up to 2,700 deaths annually (Mead et al., 1999).

The objective of this research is to develop natural ovo-antimicrobial agents as potential food preservatives which prevent or inhibit the microbial contamination caused by spoilers and pathogens found in meat products. This report focuses on the production of hyperimmunized eggs against spoilage and pathogenic microorganims, the evaluation of anti-microbial effects of specific egg yolk antibodies combined with other bactericidal or bacteriostatic egg white proteins against microorganisms for meat safety, the development of new formula of ovo-antimicrobial product.

II. MATERIALS AND METHODS

A. Bacteria and Antigen preparations

Twelve different bacteria were obtained from ATCC (U.S.A.). Twelve different bacterial cells were grown in selective liquid media and then enumerated by growing on agar plates after overnight. They were inactivated by heating at 60℃ for 30 minutes except *Clostritium perfringens* and *Bacillus cerus* which were autoclaved at 121℃ for 30 minutes. All cultures were streaked on agar plates to check the viability.

B. Immunization of chickens

All chickens were cared for in accordance with the Canadian Council on Animal Care guidelines of animal welfare. Immunization of hens was carried out as described by Sunwoo et al. (2002). Each group of heat inactivated bacterial cells was suspended in sterilized phosphate buffered saline and emulsified with an equal volume of Freund's incomplete adjuvant (Sigma, St. Louis, MO, USA). One hundred four 23-wk-old Single Comb White Leghorn (SCWL) chickens were divided into 13 cages (eight chickens per cage) and then subcutaneously immunized (1 ml of emulsion) with each group of bacterial cells (Table 1). A booster immunization was given at 2 wk after the initial immunization in the same manner. Individual eggs were daily collected and marked alphabetically for the identification purpose and stored at 4°C

until used.

C. Titer of egg yolk antibody

Specific activities of IgY in the egg yolk from laying hens hyperimmunized with bacterial cells were monitored by the indirect ELISA during the immunization period . The WSF containing IgY was assayed by an ELISA procedure as described in Sunwoo and others (1996, 2002).

D. Preparation of SpiceGuard™

Specific IgY could be obtained from egg yolks from laying hens immunized with each group of heat inactivated bacterial cells. Each group egg from hyperimmunized chickens were collected from 5 to 9 weeks and then pooled to prepare the SpiceGuard™ by spray-drying method. The flow diagram of pilot scale of SpiceGuard™ production indicates the activity of egg yolk antibody during the period of entire egg processing procedure. Approximately 333 dozens or 4,000 eggs are subjected to a series of processing steps of breaking, pasteurization, overnight cooling process, spray-drying, and packaging at the Leduc Food Development Food Processing Center (Alberta, Canada). A batch size of 165 liter of yolks was pasteurized at 62℃ for 10 min. Yield study and cost estimation to produce 80 kg of SpiceGuard™ and the antibody activity of SpiceGuard™ throughout the entire processing steps were conducted.

E. Chemical Composition

The chemical compositions and concentrations of total IgY in the SpiceGuard™ were subsequently analyzed by general chemical assay (AOAC) and the ELISA.

F. Meat preparation

Pork patties and sausage were made at the CJ Cheiljegang Foods R&D Institute (Seoul, Korea). Lean meat from the pork hams was ground through a Fujee Machinery meat grinder with a 3mm in plate. The pork back fat was cut into cubes (0.5 cm). The meat was then minced in a grinder and was thoroughly blended in a mixer. The meat block and curing ingredients including seasoning (salt, dextrose, white pepper, mace, coriander, paprika, MSG, garlic powder, sodium erythorbate; 3%), water (11%), filler (5%) and SpiceGuard™ (0, 0.1%, 0.2% or 0.5%) were mixed and stuffed into natural hog casings and then cooked in the smokehouse by slowly increasing the temperature from 40-70℃ for 2 h. The pork patties and sausages were opened in petri-dishes and vinyl bags, respective, and then stored at 10℃ and 20℃ for two weeks.

G. Bacteria determination

At each storage interval, a 20 g of meat samples from each treatment of pork patties and sausages was removed and placed in a blender containing 180ml of 0.1% peptone water (Difco Laboratories, Detroit, MI, USA) and then homogenized for 1 min. Appropriate serial dilutions were made with sterile peptone water, and 1 ml of each dilution were spread on 3M aerocount, coliform, and yeast/mold plate (3M Microbiology, Toronto, Canada). Aerobacteria and coliform plates were incubated at 35C for 48h.Yeast/Mold plates were incubated at 20C for 48h. All microbial colonies were counted and reported as log10 cfu per g of meat samples. Effects of SpiceGuard™ on the total aerobic were statistically analyzed by the procedure of the STATVIEW (SAS institute, USA). Duncan's new multiple-range test was used to compare differences among mean values ($P < 0.05$). Mean values and SEM (standard errors of the means) were reported. This experiment was replicated two times with triplicate samples in each treatment.

III. RESULTS AND DISCUSSION

Specific activities of IgY in immunized egg yolk during immunization period

Specific activities of IgY in the egg yolk from laying hens hyperimmunized with bacterial cells were monitored by the indirect ELISA during the immunization period. The result of ELISA showed that the level of specific IgY activity against bacterial cells increased from week 2 to week 4 and thereafter remained a relatively high peak, except that of specific IgY against *Staphylococcus epidermidis* (yellow line with triangle dot) and *Salmonella typhimurium* (cyan line with round dot, Fig. 1).

Chemical Composition of immunized Egg Yolk(SpiceGuard™)

The chemicals and total IgY concentrations were relatively constant among the groups regardless of the strains of bacteria as antigens. The specific IgY activity was also subsequently determined by ELISA, ranged from 0.11 to 0.71 absorbance at 405 nm (Table 1).

Effect of SpiceGuard™ on the growth of aerobic bacteria in raw pork patties and sausages during storage

Total aerobic bacteria count in pork patties during storage at 20C and 10C are shown in Table 2 and 3. The addition of highest level of SpiceGuard™ (0.5%) significantly ($p < 0.05$) reduced aerobic bacteria count in comparison without an addition of SpiceGuard™ as a control group during the storage period. The present result shows that the use of 0.5%

SpiceGuard™ can reduce the growth of aerobic bacteria in pork patties (Table 2) and sausages (Table 3) stored at 10C for 7 days by at least one log.sub.10. Therefore, the use of SpiceGuard™ at an effective antimicrobial dose can be a potential natural antimicrobial agent to retard the growth of aerobic bacteria in meat samples during the storage period. It is also evident that SpiceGuard™ can be used as a food preservative which is generally described as an inhibitor or bacteriostatic composition that simply prohibits growth in a reversible mode.

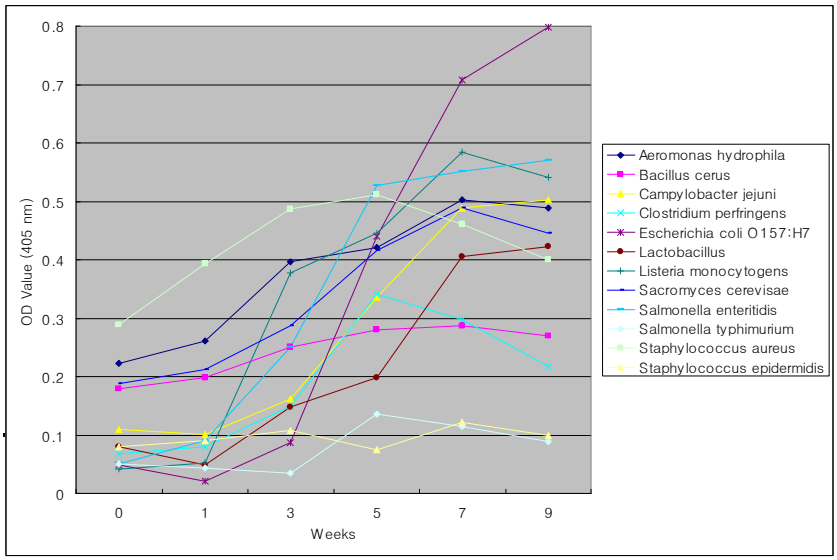

Fig. 1. The change of specific activity of IgY in the egg yolk from chickens immunized with twelve different bacterial cells which were inactivated by heat. Values are the mean of quadruple samples. Two times of immunization were conducted at 0 week and 2 week.

Table 1. Chemical composition, total IgY concentration and specific IgY profile of SpiceGuard™.

Composition	**% in SpiceGuard™**
Moisture	3.2%
Protein	49.1%
Fat	42.3%
Carbohydrate	0.9%
Ash	4.5%
Total IgY	15.5mg / g
Specific IgY	Titer (absorbance at 405 nm)
Aeromonas hydrophila	0.502
Bacillus cerus	0.287
Campylobacter jejuni	0.488
Clostridium perfringens	0.297
Escherichia coli O157:H7	0.707
Lactobacillus	0.404
Listeria monocytogens	0.584
Sacromyces cerevisae	0.489
Salmonella enteritidis	0.552
Salmonella typhimurium	0.115
Staphylococcus aureus	0.461
Staphylococcus epidermidis	0.122

Table 2. Effect of SpiceGuard™ on total aerobic bacteria(log_{10} CFU / g) of pork patties during storage at 10℃ and 20℃.

Day	**0.5%**	**0.2%**	**0.1%**	**Control**
10℃				
0	2.87 ± 0.12^{a}	2.90 ± 0.04^{a}	2.98 ± 0.02^{ab}	3.10 ± 0.09^{b}
7	6.98 ± 0.03^{a}	7.30 ± 0.03^{b}	7.38 ± 0.05^{b}	7.56 ± 0.03^{c}
14	9.88 ± 0.04^{a}	10.44 ± 0.04^{b}	10.72 ± 0.07^{c}	10.98 ± 0.11^{d}

20℃				
0	2.87±0.12[a]	2.90±0.04[a]	2.98±0.02[a]	3.10±0.09[a]
3	7.22±0.03[a]	7.38±0.03[ab]	7.41±0.04[bc]	7.49±0.03[bc]
6	8.23±0.07[a]	8.42±0.06[ab]	8.56±0.08[bc]	8.60±0.08[bc]

[a–c] Means ± SEM in the same raw with different superscripts are significantly different ($P < 0.05$).

Table 3. Effect of SpiceGuard^{TM} on total aerobic bacteria (log_{10} CFU / g) of sausages during storage at 10℃ and 20℃.

Day	**0.5%**	**0.2%**	**0.1%**	**Control**
10℃				
0	2.74±0.06[a]	2.87±0.04[ab]	2.87±0.02[ab]	2.89±0.02[b]
7	5.25±0.22[a]	5.99±0.06[ab]	6.17±0.06[ab]	6.45±0.08[b]
14	7.34±0.08[a]	7.77±0.04[b]	7.88±0.10[b]	7.89±0.04[b]
20℃				
0	2.74±0.06[a]	2.87±0.04[ab]	2.87±0.02[ab]	2.89±0.02[b]
3	5.60±0.14[a]	5.82±0.04[a]	5.97±0.06[a]	5.92±0.11[a]
6	7.62±0.08[a]	7.78±0.04[ab]	7.95±0.04[bc]	7.96±0.04[c]

[a–c] Means ± SEM in the same raw with different superscripts are significantly different ($P < 0.05$).

IV. CONCLUSION

New demands of food manufacturers focus attention on the safety of raw and processed meat. The microbial spoilage is one of major challenge to the food processing industry. The effective growth inhibition of microbial contamination is the first important step to prevent food spoilage. Contamination of sausage by microorganisms can occur with excessive handling during the processing. There is immediate issues to produce safe meat products that can extend shelf life during long periods of time without spoilage and that can remove food-born pathogens by using a natural antimicrobial agent without causing side effects.

SpiceGuard^{TM} could be considered as a potential food additive and/or food preservative to prevent bacterial growth on the surface of food before packaging or consuming. The advantage of SpiceGuard^{TM} is that it is a 100% natural, non-chemical, and non-toxic food ingredient. SpiceGuard^{TM} is designed to replace antibiotics and address the consumer's growing demand for natural food preservatives. SpiceGuard^{TM} is proved to prevent microbial growth for both gram negative and gram positive. Plus showed activity in high acid environment, up to pH 3, as well as high temperature, up to 75°C.

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