EFFICACY OF COUMARIN AND (-) EPICATECHIN ON SALMONELLA REDUCTION AND SHELF LIFE ENHANCEMENT IN CHICKEN LIVER PRODUCTS

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Abstract – Edible variety meats such as chicken livers are highly perishable and have the potential to be contaminated with *Salmonella*. The present study was designed to determine the inhibitory effects of natural plant compounds coumarin (C), (-) epicatechin (E) and their combination (CE) on the survival of *Salmonella* spp. on chicken livers. Fresh and cooked ground chicken liver samples were inoculated (10^6 CFU/ml) with a cocktail of *Salmonella* spp. (*S. paratyphi*, *S. choleraesuis, S. typhimirium* and *S. heidelberg*) and packaged in polypropylene containers (PPC) or polyethylene vacuum-seal bags (PEVB) and stored at 4°C for 30 days. The shelf life and quality attributes of the products were evaluated weekly using microbiological (bacterial counts) and chemical (water activity and pH) analyses. Results indicated that CE (200 ppm) inhibited the growth of *Salmonella* in fresh chicken livers by 5 and 3.5 log CFU/g and inhibited the cooked chicken liver samples by 2 and 1.5 log CFU/g in the PPC and PEVB, respectively. In addition, the shelf-life of fresh and cooked chicken liver products could be extended in excess of 30 days by utilizing CE at 200 ppm.

Key Words – Antimicrobial activity, Inhibition, Phenolic compounds

I. INTRODUCTION

Chicken liver and its products are variety meats, a rich and economical source of essential nutrients, and prone to rapid microbial spoilage as well as a high incidence of pathogenic microorganisms [1].

Salmonella is one of the significant foodborne pathogens present in raw poultry and meat products which is responsible for large number of life threatening foodborne diseases [2, 3, 4].

Although the food industry is employing several food safety practices, only a few of them have been adapted by the poultry and/or fresh meat industry due to their quality decline, possible health risks, consumer conflict and cost implications [3]. Thus, consumers still have concerns about the health problems caused by foodborne pathogens [5, 6] and the demand for the alternative strategies to prolong the shelf life and enhance the food safety of products using natural antimicrobials and/or preservatives is increasing [4, 6, 7].

Previous studies on plant based compounds have focused on meat livers and the majority of these have not been conducted on chicken liver. Recently, efficacy of plant based components was reported to inhibit the growth of *Salmonella* up to a significant reduction in fresh meat and poultry products [4, 8, 9] by maintaining their shelf life [10].

According to Cetin-Karaca [11], the natural bioactive phenolic compounds including coumarin (C) and (-) epicatechin (E) have great potential as antimicrobial food ingredients due to their generally recognized as safe (GRAS) status and desirable organoleptic characteristics such as; low odor and no color. Furthermore, storage parameters (time, temperature, and types of packaging), and product parameters (pH, water activity, and fat content) on the efficacy of novel applications require further study. The overall goal of this study is to evaluate the antimicrobial efficacy of C and E against *Salmonella* to enhance the food safety and the shelf life of chicken livers while investigating their effects on storage time and packaging systems; polypropylene containers (PPC) and polyethylene vacuum-seal bags (PEVB).

II. MATERIALS AND METHODS

Preparation of bacteria and bioactive compounds

Four strains of pathogenic *Salmonella* spp. were used including; *S. paratyphi* (UK-Micro 29A), *S. cholerasuis subsp. cholerasuis* (ATCC 10708), *S.*

typhimirium (ATCC 13311), and *S. heidelberg* (biocontrol S-67, + control). Culture cells were grown at 37°C and maintained on slants of brain-heart infusion (BHI) agar, and stored at 4°C until needed.

Two concentrations; 100 and 200 ppm (µl/L) of each phenolic compound were prepared in ethanol (95%). The final phenolic solution was adjusted to approximately pH 5.00 to ensure the pH would not affect the bacterial growth. The solutions were filter sterilized using 0.2 µm filters.

Inoculation, application and packaging of samples

Chicken livers were supplied from a local retail store. Half of them were boiled in stainless steel containers to an internal temperature of 165F (76°C) and pureed in a lab grade sterile blender. Inoculation for fresh chicken livers (FCL) was performed by immersing them for 5 minutes in a cocktail of four *Salmonella* strains (10^8CFU/ml) and batch inoculation (10^6CFU/ml) and hand massage was applied to cooked chicken livers (CCL) for even distribution. Inoculated samples were dried under the bio safety hood for 30 minutes at room temperature to allow *Salmonella* attachment.

Samples were randomly divided into groups and then, each FCL group was immersed in 1L of either 100 or 200 ppm (µl/L) of C, E or CE (50% v/v). After the treatments, chicken livers were drained for 15 minutes at room temperature. Pureed CCL were hand massaged for 5 minutes with 10 ml of each treatment. Non-treated samples served as controls (A). Both FCL and CCL were packaged (40 ± 5 g/package) aseptically in polyethylene sterile bags with 80% vacuum and polypropylene sterile containers with lids. Samples were stored at 4°C for 30 days.

Microbiological and chemical analyses

Chicken livers (20 ± 2 g each) from each packaging system were added to 180 ml of sterile 0.10% (w/v) peptone water (PW) in a sterile stomacher bag and homogenized for 1 minute in a stomacher. The pH, water activity (a_w) and bacterial growth were determined at 0, 1, 7, 15, 21 and 30 days of storage. Appropriate dilutions in PW were surface plated on XLD agar to quantify *Salmonella* population. After incubation at 37°C for 24 hours, viable colonies were counted and reported as log10 CFU/g of the sample. Experiments were replicated twice with different liver samples and analyses were run in duplicates for each replicate.

III. RESULTS AND DISCUSSION

Each of the treatments was observed to be highly effective for inactivation of *Salmonella*, confirming natural plant compounds are effective to control *Salmonella* and other pathogens [4, 9, 13]. However, the 100 ppm treatments were observed to be ineffective after day 15 in FCL and the product quality had become unacceptable. Therefore, the control groups and the 100 ppm treatments were not eligible for extended storage. Previously, shelf life of FCL was extended using thyme oil [14] and oregano oil [15] along with different packaging systems. Accordingly, both FCL and CCL treated with higher concentrations (200 ppm) of the phenolic compounds were observed to have an extended shelf life (30 days or more) while maintaining their sensory characteristics. They did not show any signs of spoilage such as; off-odor, discoloration or deterioration in texture. CE (200 ppm) showed the highest inhibition in *Salmonella* among the all treatments; by 5 and 3.5 log CFU/g (Fig 1a; 1b) in FCL and by 2 and 1.5 log CFU/g (Fig 1c; 1d) in CCL samples in PPC and PEVB, respectively.

The pH and a_w of treated samples and controls over 30 days of storage time were depicted in Table 1 and Table 2 for FCL and CCL, respectively.

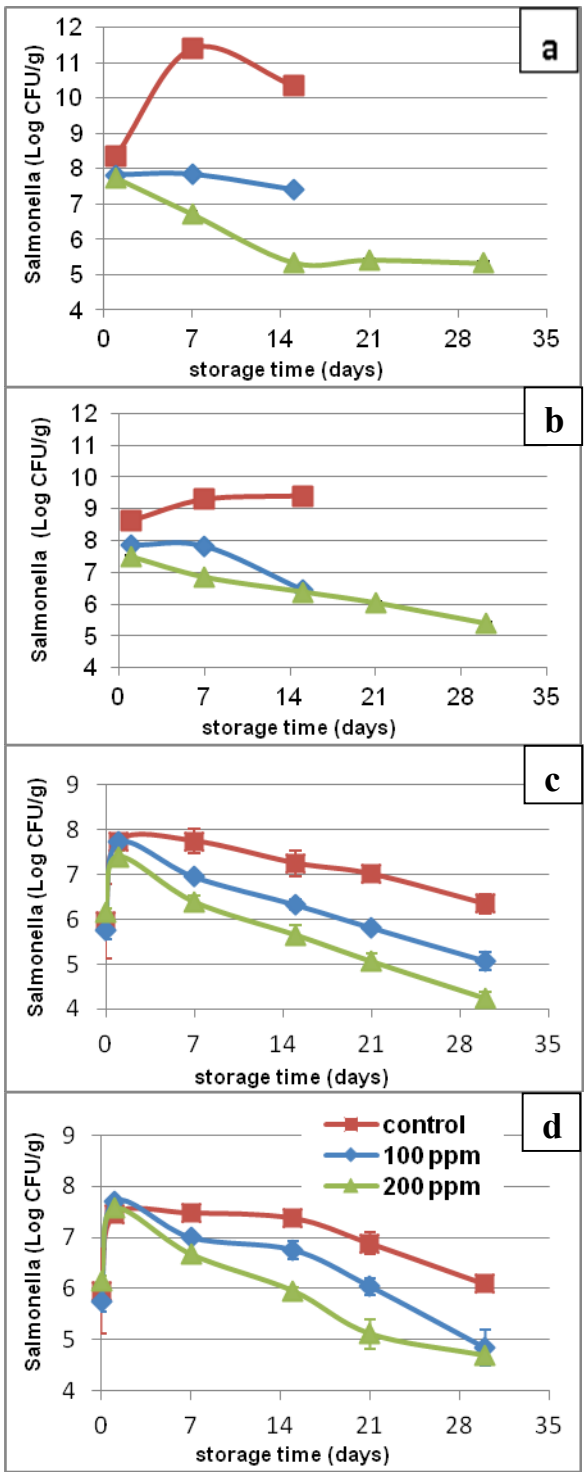

Figure 1. Antimicrobial efficacy of CE against *Salmonella* spp. in FCL stored in PPC (a) and PEVB (b) and CCL stored in PPC (c) and PEVB (d). Each point is the mean of two samples taken from 2 replicates (n=2x2=4). Error bars represent standard deviation from the mean, P <0.05.

Table 1 pH and a_w of FCL treated with C, E and CE in PPC and PEVB.

Treatment	Analyses	Storage time (day)	
PPC		1	30
Control	pH	6.87±0.01a	NA
	a_w	0.91±0.01a	NA
Coumarin	pH	6.77±0.01a	5.66±0.04a
	a_w	0.89±0.08a	0.97±0.05a
(-) Epicatectin	pH	6.88±0.02a	5.84±0.01a
	a_w	0.88±0.03a	0.98±0.01a
Combination	pH	6.69±0.01a	5.35±0.08b
	a_w	0.93±0.01a	0.98±0.01a
PEVB		1	30
Control	pH	6.82±0.01a	NA
	a_w	0.90±0.02a	NA
Coumarin	pH	6.77±0.06a	5.36±0.05a
	a_w	0.94±0.01b	0.97±0.02a
(-) Epicatectin	pH	6.68±0.01b	5.56±0.04a
	a_w	0.93±0.02a	0.95±0.06a
Combination	pH	6.70±0.03a	5.64±0.01b
	a_w	0.92±0.05a	0.95±0.01a

NA: not available
Mean values ± standard deviations (n = 4) within the same column with the same letter are not significantly different (P < 0.05)

Table 2 pH and a_w of CCL treated with C, E and CE in PPC and PEVB.

Treatment	Analyses	Storage time (day)	
PPC		1	30
Control	pH	6.11±0.02a	6.61±0.01a
	a_w	1.00±0.04a	1.03±0.07a
Coumarin	pH	6.28±0.05a	6.78±0.06b
	a_w	0.99±0.01a	1.01±0.02a
(-) Epicatectin	pH	6.35±0.07b	6.63±0.08a
	a_w	0.99±0.02a	1.01±0.02a
Combination	pH	6.38±0.01b	6.77±0.06b
	a_w	0.99±0.07a	1.01±0.01a
PEVB		1	30
Control	pH	6.84±0.07a	6.56±0.02b
	a_w	1.00±0.03a	1.00±0.01a
Coumarin	pH	6.84±0.02a	6.80±0.09a
	a_w	0.98±0.01b	1.00±0.04a
(-) Epicatectin	pH	6.85±0.03a	6.62±0.05b
	a_w	1.00±0.09a	1.00±0.06a
Combination	pH	6.85±0.06a	6.84±0.02a
	a_w	0.99±0.02a	1.00±0.03a

Mean values ± standard deviations (n = 4) within the same column with the same letter are not significantly different (P < 0.05)

During the extended storage time all the fresh samples exhibited a slight decrease (from 6 to 5) in pH, while there wasn't a significant pH change in cooked samples. Since the compounds were adjusted to approximately pH 5 prior to treatments, the pH drop in FCL during the storage was mainly caused by the liver's high glucose level, the bacterial growth (spoilage) and extended storage time. There wasn't a significant change in a_w of all samples.

IV. CONCLUSION

This study is the first to demonstrate the antimicrobial efficacy of C and E against pathogenic *Salmonella* in chicken livers. For both packaging systems; PPC and PEVB, antimicrobial activity of CE was significantly greater when it was used at 200 ppm. Not only the higher concentrations of these bioactive compounds inactivated *Salmonella*, they also prolonged the shelf life of chicken livers up to 30 days while maintaining their sensory characteristics. Ultimately, the use of natural phenolic compounds including the combination of C and E provides synergistic effect and additional safety and improve the shelf life of chicken livers. Therefore, they have a potential to be substituted with chemical preservatives to increase shelf life in poultry products. However, further research should be conducted to determine their interactions with other food ingredients.

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