EFFICACY OF LACTIC ACID AND SODIUM BENZOATE AS WASH SOLUTIONS IN PREVENTING RECONTAMINATION OF SOME SPECIFIC MICROBES ON REFRIGERATED CHICKEN CARCASS

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Abstract
An experiment was conducted on the efficacy of lactic acid and sodium benzoate as wash solutions in preventing recontamination of some specific microbes on refrigerated chicken carcass. 50g of frozen chicken wings were washed in wash solutions of 0.1 % sodium benzoate (Treatment A) and 0.1 % sodium benzoate + 0.4 % lactic acid (Treatment B). Microbial load were counted at days 0 (before refrigeration), 3, 6 and 9. Data was arranged using a 2 x 4 factorial formats and analyzed using a two way analysis of variance. All parameters measured were significantly ($P<0.05$) different except for fungi. Results on chicken carcass washed with both treatments A and B showed the same trend of microbial load in which all the bacteria were reduced up to day 3 of refrigeration. Increase in microbial load was observed after day 3 of refrigeration up till day 6 and decreased again at day 9. Staphylococcus aureus which had an initial microbial count of 192 log_{10}cfu/ml before washing in treatment A, increased up to 270 log_{10}cfu/ml at day 6 of refrigeration and later reduced to 224.5 log_{10}cfu/ml at day 9 of refrigeration. However, the combined solution (treatments B) reduced Staphylococcus aureus to 4.5 log_{10}cfu/ml at day 3. But an increase was observed at day 6 (8 log_{10}cfu/ml) and later decreased at day 9 of refrigeration (4.5 log_{10}cfu/ml). Both treatments totally reduced all fungi at day 9 of refrigeration to 0 log_{10}cfu/ml. Although both washing solutions can reduce microbial load on chicken if refrigerated, treatment B effectively reduce more microbial population of refrigerated chicken carcass than treatment A.

Keywords: Lactic acid, Sodium Benzoate, *Staphylococcus aureus* and Refrigerated Chicken Carcass

Introduction

Meat is an excellent source of high quality protein which contains vitamins and certain minerals especially iron. Meat also supplies nutrients which contribute significantly to the dietary balance of meals. Some meats are being contaminated with bacteria such as (*Salmonella spp. Shigella spp. Escherichia coli and Staphylococcus)* and fungi such as (*Rhizopus microporus, Penicillum solitum, Aspergillus flavus, Saccharomyces spp*). Chemicals that can be used to suppress these bacteria and fungi growth include, lactic acid solution, sodium benzoate solution while other preservative measures such as salting, refrigerating, canning, roasting can be used. Lactic acid is a water-soluble liquid that freezes, or solidifies, at 64.4°F (18°C) just slightly below normal room temperature. Sodium benzoate is a preservative, bacteriostatic and fungistatic under acidic conditions. It is used most prevalently in acidic foods such as salad dressings (vinegar), carbonated drinks (carbonic acid), jams and fruit juices (citric acid), pickles (vinegar), and condiments. Studies have been made using the hazard analysis and critical control point (HACCP) principles (Ropkins and Beck, 2000) of intervention system in reducing bacterial load on animal product (Amymerich *et al.*, 2008; Bolton *et al.*, 2001; Koohmarine *et al.*, 2005 and Sanwo *et al.*, 2009), therefore this study aimed at determining the efficacy of lactic acid and the combined action of sodium benzoate and lactic acid as wash solutions in preventing recontamination of some specific microbes on refrigerated chicken carcass.

Materials And Methods

***Experimental sites*:** The first phase of the experiment which was washing (by agitation) of meat was carried out at meat science laboratory of the Department of Animal Production and Health (APH) while microbial load counting and identification was carried out at the Department of microbiology, University of Agriculture, Abeokuta, Ogun State.

Materials used: Sterile bottles, knife, methylated spirit, cotton wool, trays, wire loop, spirit lamp, conical flask, petri-dish, MacCartney bottles, slides and cover slips, weighing balance, autoclave, incubator, stomacher blender, Mannitol salt agar, potato dextrose agar, deoxycholate citrate agar, MacConkey agar, plate count agar, petri-dish.

Experimental Procedures

First phase (washing of meat at different treatment levels): Frozen Chicken wings were purchased from Kuto market in Abeokuta Ogun

Table 1 : The main effect of chemical and refrigeration days on microbial population on chicken carcass

	CHEMICALS (log_{10}cfu/ml)		REFRIGERATION DAYS			
Microbes	0.1% sodium benzoate	0.1% sodium benzoate + 0.4% lactic acid	0	3	6	9
Staphylococcus	161.38 ± 105.26^{a}	9 ± 6.93^{b}	17.5 ± 3.0^{b}	69.75 ± 76.34^{ab}	139 ± 153.26^{a}	114.5±127.14^{ab}
Esherichia coli	261.25 ± 65.79^{a}	144.88 ± 103.02^{b}	172.25 ± 135.98^{cb}	115± 46.37^{c}	225 ± 86.60^{ab}	300 ± 02^{a}
Salmonella shigella spp	185.63 ± 125.36	149.25 ± 130.48	273.5 ± 15.19^{a}	35.5 ± 37.50^{b}	70.75 ± 75.24^{b}	290 ± 12.54^{a}
Asper Flavus	2.88 ± 3.87	3.125 ± 3.48	5.75 ± 2.21^{a}	0^{b}	6.25 ± 3.20^{a}	0^{b}
Asper niger	0.125 ± 0.35	0.38 ± 0.52	0.5 ± 0.58	0	0.5 ± 0.58	0
Geotrichum	5.88 ± 14.70	2.88 ± 4.55	4.75 ± 4.65^{a}	0^{b}	19.96 ± 9.98^{b}	0^{b}
Candida	24.38 ± 36.83	6.00 ± 8.33	47.75 ± 37.70^{a}	0^{b}	13 ± 17.93^{b}	0^{b}
TPC	263.75 ± 67.18	206.75 ± 99.71	190 ± 127.03^{a}	155 ± 5.77^{b}	300± 02^{a}	296 ± 8.02^{a}

^{a,b} Means along the same row with different supercripts are significantly different (P<0.05)
TPC = Total Plate Count

Table 2: The interactive effect of organisms, days and treatments on chicken carcass

	0.1% SODIUM BENZOATE (log_{10}cfu/ml)				0.1% SODIUM BENZOATE + 0.4% LACTIC ACID (log_{10}cfu/ml)			
Microbes	0	3	6	9	0	3	6	9
Staphylococcus	16 ± 0^{d}	135± 21.21^{c}	270± 42.43^{a}	224.5 ± 9.19^{b}	19 ± 4.24^{d}	4.5 ± 2.12^{d}	8 ± 4.25^{d}	4.5 ± 3.54^{d}
Escherichia coli	290 ± 2.83^{b}	155 ± 7.07^{c}	300 ± 0^{a}	300 ± 02^{a}	54.2 ± 2.12^{e}	75 ± 03^{d}	150 ± 03^{c}	300 ± 02^{a}
Salmonella shigella spp	286.5 ± 3.54^{a}	52 ± 53.74^{b}	104 ± 107.48^{b}	300± 0^{a}	260.5 ± 2.12^{a}	19 ± 15.56^{b}	37.5 ± 31.82^{b}	280 ± 8.49^{a}
Aspergillus flavus	4 ± 1.14^{ab}	0^{b}	7.5 ± 4.95^{a}	0^{b}	7.5 ± 0.71^{a}	0^{b}	5 ± 02^{a}	0^{b}
Aspergillus niger	0.51 ± 0.71^{ab}	0^{b}	0^{b}	0^{b}	0.5 ± 0.71^{ab}	0^{b}	1 ± 02^{a}	0^{b}
Geotrichum	2.51 ± 3.54	0	21 ± 29.70	0	7 ± 5.66	0	4.5 ± 6.36	0
Candida	78.5 ± 21.92^{a}	0^{b}	19 ± 26.87^{b}	0^{b}	17 ± 01^{b}	0^{b}	7 ± 9.90^{b}	0^{b}
TPC	300 ± 02^{a}	155 ± 7.07^{b}	300 ± 02^{a}	300 ± 02^{a}	80 ± 2.83^{c}	155 ± 7.07^{b}	300 ± 02^{a}	292 ± 11.31^{d}

^{abcd} Means along the same row with different supercripts are significantly different (P<0.05)
TPC = Total Plate Count

state. 50 grams skin samples from the frozen chicken wings were removed and subjected to solutions of 0.1 % sodium benzoate (treatment A) and 0.4 % of combined sodium benzoate lactic acid (treatment B) which were prepared by diluting the percent solution in 100 ml distilled water. Each skin sample was washed in recommended solution and replicated thrice for each treatment. Samples were washed at constant speed for 10 sec by agitation in a stomacher laboratory blender. Each of the washed samples was then refrigerated at 4°C and samples were taken at days 0, 3, 6 and 9 for the identification of some specific microbes and total plate count.

Second phase (Microbial Load Counting and Identification): The Serial-dilution process

Samples were then collected and thoroughly mixed in order to have micro organisms evenly distributed. Test tubes containing 9 ml sterile distilled water as diluents were set up. 1ml of each sample was weighed into diluents and a sterile pipette was set to transfer 1 ml of the sample serially into a test tube using sterile pipette at each step.

***The pour plate count*:** The plating of samples was done by using the 'pour plate' technique. 1 ml of the diluents from the test tubes was poured onto the diluents in the petri dishes and it was allowed to wet set before the plating was incubated.

The media used were Mannitol salt Agar (MSA), MacConkey Agar (MA), and Plate Count Agar (PCA), Deoxycholate citrate Agar (DCA), Potato Dextrose Agar (PDA), were poured into the Petri dishes in order to identify *Staphylococcus aureus, Escherichia coli, Salmonella Shigella spp,* and fungi such as *Aspergillus flavus, Candida spp, Rhodotorula spp etc.* Potato Dextrose Agar media was used for the fungi and Plate Count Agar were used for Total Plate Count.

Sterilization of Glass Wares: Disposable petri dish were used and arranged in the canister, while pipette and other glass containers were washed, drained, dried and wrapped with an aluminum foil and sterilized. After sterilization, the petri dishes were removed alongside with other glass wares and allowed to cool in readiness for use. Work surfaces were cleaned by sterilizing with methylated spirit. Aseptic working environment

was achieved with the use of spirit lamp flame.

Total Bacteria count (cfu/ml)

The total bacteria count was determined with the pour plate technique using the plate count agar. The plates were incubated between 24 hours at 37 ^{0}C. All colonies appearing at the end of the incubation period were counted and the counts were expressed in colony forming unit per gram of the sample. Colonies of bacteria developing on the plates were observed, isolated and re-isolated on a fresh media until pure culture was obtained.

Identification of isolates: The bacteria isolated were then identified using both morphology culture characteristics, i.e colour, consistency, shape, size, elevation, edge, opacity and biochemical test (such as oxidase, citrase, catalase etc). Bacteria were identified base on the result obtained from biochemical characterization.

Data collection

After the incubation periods, the number of colonial growth and specific growth were counted visually and recorded in colony forming unit per gram (log 10cfu/ml) on peptone-saline wash solutions for each treatment. Some specific microbes that were identified are; *Salmonella Shigella spp, Staphylococcus aureus, Escherichia coli* and total plate count were done.

Statistical Analysis: Data collected were subjected to analysis of variance (ANOVA) in a 2 x 4 factorial arrangement (SAS), using Randomized Complete Block Design.

Results and Discussion

The value obtained for treatments as shown on Table 1 was the effectiveness between the two chemicals used. The effect of combined 0.1% sodium benzoate + 0.4% lactic acid was more effective in getting rid of most of the bacteria and fungi identified. The combined chemicals of 0.1% sodium benzoate + 0.4% lactic acid was not effective in reducing growth of some fungi such as *Aspergillus flavus* and *Aspergillus niger.* Also there was reduction of the microbes especially at days 0 and 3, while there was an increase of the microbes as the days increases especially at day 6.. Both wash chemicals effectively reduced microbial load of *Escherichia coli* and *Salmonella shigella spp* up to day 3 of refrigeration. *Staphylococcus aureus* showed a different pattern in which both chemicals could not reduce the microbial population at the 9th day of refrigeration which agrees with the findings of Chung (1988) who reported a significantly reduced bacteria load of *Salmonella shigella spp.* Table 2 shows the interactive effect of treatments (wash chemicals) and refrigeration days on washed chicken carcass. In general, refrigeration is supposed to reduce the action of the microbes but Table 2 shows a fluctuating growth which may be due to unstable power supply which probably influenced increased in microbial population. However, 0.1% sodium benzoate and combined 0.1% sodium benzoate + 0.4% lactic acid are effective in reducing microbial population of refrigerated chicken.

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