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The Development of Microbial Count, Psychrotrophic Count, Odor, and Catalase Activity in Chicken Meat Treated with Organic Acid During Shelf Life at Refrigerated Storage

تطور نمو العدد الكلي للأحياء الدقيقة والمحبة للبرودة والتزنخ ونشاط أنزيم الكاتاليز في لحوم الدواجن التي عوملت بفوسفات ثلاثي الصوديوم وحامض اللاكتيك وحامض الخليك خلال فترة التخزين المبرد في الثلاجة

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Abstract: This study was conducted to evaluate the shelf life of chicken meat after treatment with 10% trisodium phosphate (TSP), 1% lactic acid (LA), and 0.5% acetic acid (AA) solutions. Total count, psychrotrophic count, off-odor, and catalase activity were examined. Chicken carcasses were sprayed with TSP, LA, AA, for 10 seconds and stored at 4±0.5°C for 15 days. Total count of chicken surface control reached 6.5 log CFU/g after 7 days while it reached 5.8, 5.5, and 6.2 log CFU/g in, 10% TS, 0.5% AA, and 1% LA after 13 days of storage, respectively. Total count and psychrotrophic count were significantly lower ($P<0.05$) for chicken samples treated with LA, AA and TSP than the control samples throughout the entire storage period. Sensory acceptability limit (off-odors) was reached for the control chicken samples on the day 7th, on day 13th for the samples treated with LA, and on day 15th for TSP, and AA treated samples. The number of the catalase positive colonies increased in the control from 78% to 93% within 7 day; also it increased from 70% to 90% in treated samples within 7 day. At the end of the storage period, the effectiveness of TSP and AA was higher than that of LA. The use of TSP, AA, and LA extended the shelf-life period 8, 8, and 6 days for chicken meats, respectively.

Keys word: *shelf life; chicken; trisodium phosphate; acetic acid; lactic acid count; catalase activity.*

المستخلص: فأجريت هذه الدراسة لتقييم فترة صلاحية لحوم الدواجن بعد معاملتها بفوسفات ثلاثي الصوديوم بنسبة 10 %، وحامض اللاكتيك بنسبة 1 % ، وحامض الخليك بنسبة 0.5 % . وقد تم العد الكلي للأحياء الدقيقة والمحبة للبرودة، والتزنخ، ونشاط انزيم الكتاليز في اللحوم المعالجة والعينة الضابطة. عولجت لحوم الدواجن عن طريق رشها بهذه الاحماض لمدة عشر ثواني وبعد ذلك تم وضعها في عبوات بلاستيكية معقمة وتخزينها في المبرد 4 ± 0.5 درجة مئوية لمدة 15 يوما. تم العد الكلي للأحياء الدقيقة من على سطوح لحوم الدواجن وقد بلغ لوغاريثم العدد الكلي في العينة الضابطة 6.5 بعد 7 أيام في حين وصلت إلى 5.8، 5.5، و 6.2 في العينات المعالجة بفوسفات ثلاثي الصوديوم بنسبة 10 %، وحامض اللاكتيك بنسبة 1 %، وحامض الخليك بنسبة ٪0.5 بعد 13 يوما من التخزين، على التوالي. ولقد حدثت تغيرات واضحة ($p0.05 >$) في العدد الكلى للأحياء الدقيقة في اللحوم المعالجة بفوسفات ثلاثي الصوديوم بنسبة 10 % وحامض الخليك بنسبة ٪0.5 وحامض اللاكتيك بنسبة 1 % مقارنة بالعينة الضابطة. وقد تم ملاحظة التزنخ والروائح غير المرغوبة في العينة الضابطة في اليوم السابع وكذلك في اليوم 13 للعينة المعالجة بحامض اللاكتيك بنسبة 1 % وبعد 15 يوم في العينات المعالجة بفوسفات ثلاثي الصوديوم بنسبة 10 %

وحامض الخليك بنسبة 0.5 %. كما تم عد المستعمرات الإيجابية لإنزيم الكاتليز ولوحظ زيادة في عدد الموجبة للأنزيم ولوحظ زيادة من 78 % في العينة الضابطة إلى 93 % بعد مرور 7 ايام في المبرد، كما انها زادت من 70 % إلى 90 % في العينات المعالجة بفوسفات ثلاثي الصوديوم بنسبة 10 %، وحامض اللاكتيك بنسبة 1 %، وحامض الخليك بنسبة ٪0.5 بعد مرور 7 ايام في المبرد. في نهاية فترة التخزين كانت فعالية فوسفات ثلاثي الصوديوم بنسبة ٪10 وحامض الخليك بنسبة ٪0.5 اكثر فاعلية من حامض اللاكتيك بنسبة 1 %. وقد أوضحت النتائج أن استخدام فوسفات ثلاثي الصوديوم بنسبة 10 % وحامض الخليك بنسبة 0.5 % وحامض اللاكتيك بنسبة 1 % ادت الى زيادة فترة الصلاحية بـــ 8، 8، و 6 أيام للحوم الدواجن على التوالي.

كلمات مدخلية : *فترة الصلاحية، الدجاج، ثلاثي فوسفات الصوديوم، حامض الخليك، قياس حامض اللاكتيك، نشاط انزيم الكتاليز.*

INTRODUCTION

Microbial quality of raw chicken meat is a good indicator for determining the level of contamination in chicken meat during slaughtering, handling and packaging. Psychrotrphic and mesophilic pathogens could grow in both temperature abuse and during extended refrigerated storage (Marth, 1998). In 2007, The Japanese Ministry of Health, Labour, and Welfare reported that more than 40 causative agents of food-borne illnesses were caused by consumption of chicken meat and chicken meat by-products which were contaminated by *Campylobacter* spp. (*Suzuki and Yamamoto, 2009*). Several techniques have been applied to decontaminate chicken meat surface from pathogenic and non pathogenic bacteria. Aerobic total count in chicken meat is often used as an indicator for the quality of meat. The initial micro-flora in meat is mainly mesophilic after the carcass evisceration and count can reach 10^2 to 10^4 CFU/cm^2 (Dainty and Mackey, 1992). During storage at the refrigerator temperature, the psychrotrphic bacteria develop and dominate. *Pseudomonas* spp. constitutes 50 to 90% of the total aerobic bacteria in meat due to their growth at low temperature with short generation time as compared to the other microorganisms (Stiles, 1991). The majority of spoilage microorganisms in meat can grow between 0-10°C (Jay, 1992). Therefore, with low temperature storage combined with antimicrobial treatments to lower microbial load the shelf life can be increased and meat safety improved. *Campylobacter jejuni, Campylobacter coli, Escherichia coli O157:H7, Staphylococcus aureus, Salmonella* spp., *and Listeria monocytogenes* are the main pathogens which were isolated from chicken and their initial load *can be significantly decreased by* trisodium phosphate and citric acid treatment *(Suzuki and Yamamoto, 2009 and* Basfar *et al*., 2007). However, the genus *Pseudomonas* is responsible for spoiling meat under aerobic conditions and at low temperature (Kraft, 1992).

Acetic acid (E260) and Lactic acid (E270) have been used as preservatives in many types of food (Doores, 1990). Trisodium phosphate (TSP) (E339) is a food additive and is used as an acidity regulator emulsifier, and thickening agent (Codex, 2007, and Watson, 2002). The U.S. Deptartment of Agriculture has approved the use of TSP in poultry processing to eliminate *Salmonella* contamination. A 7.5% TSP concentration can influence the growth of psychrotrophic and *Enterobacteriaceae* in chicken meat breasts (Sallam and Samejima, 2004).

Off-Odor develops in meat as spoilage proceeds, and this leads to rejection by the consumers. It is mainly due to oxidation and growth of microorganism, and occurs as a result of enzymatic and bacterial processes. Oxidation of lipids in chicken meat is responsible for the odor and changes in nutritional quality (vitamins and amino acid), flavor, taste and texture (Aguirrezabal *et al*., 2000). Oxidation can be initiated and accelerated by oxygen or light. Off-odor or rancidity reduces the shelf life and as a result it affects the meat industry. Antioxidants addition can stop or reduce the oxidation process in meat and meat products and spoilage organisms.

Catalase enzyme is present in aerobic and some facultative microorganisms. Psychrotrophs which are responsible for spoilage of many aerobically cold-stored foods possesses high catalase activity. Bacteria are considered catalase positive when bubbles are generated on the surface colonies after application of 3% hydrogen peroxide. Catalase negative do not

generate bubbles on the colony surface. Catalase test can be used to monitor the change of micro flora during storage of meat and meat products.

The purpose of this study was to evaluate the shelf life of chicken meat as affected by 10% (TSP), 1% lactic acid (LA) and 0.5% acetic acid (AA) solution during storage at 4±0.5°C. In addition, off-odor and catalase activity were also examined.

MATERIALS AND METHODS

Materials

Whole chicken meat bought from local market, on the same day of production. Plate count agar for Total count and Psychrotrophic count

Tri-sodium phosphate: Molecular formula: Na_3HPO_4. $12H_2O$, Molecular weight: 380.12, CAS No.: 7758-79-4, Standard Executed: HG/T 2517-93, Specifications Industrial Grade Food Grade, Main content %≥ 98.0.

Lactic acid: Food grade DL-lactic acid, Molecular Formula: $C_3H_6O_3$, Structural Formula: $CH_3CHOHCOOH$, Molecular Weight: 90.08, Specifications: 80%.

Acetic acid: Glacial Acetic Acid 99%99.5 & . Tech grade & food grade. Purity: 99%.

3% Hydrogen Peroxide Topical Solution U.S.P: Hydrogen peroxide food grade, CAS No.: 7722-84-1, Molecular Weight: 34.01, Chemical Formula: H_2O_2 in aqueous solution (3%), Product Codes: 2180, 2182

Methods

Treatment of Meat

Thirty six whole chicken were obtained from a local market and transported to the laboratory and used within 1 hour of purchase. Whole chicken was weighted in the laboratory and its weight was about 750±50g. A solutions of TSP (10%), LA (1%), and AA (0.5%) was prepared. Distilled water was used as solvent. Whole chicken meat was aseptically sprayed on the surface by hand held sprayer and left for 5 seconds. Then, chicken meat was packed in stomacher bag and stored at 4°C±0.5°C. This experiment was repeated three times using thirty six of whole chicken meat, each time.

Microbial Counts

On each sampling time (0, 1, 3, 5, 7, 9, 11, 13, and 15 days), 25 g of chicken meat (leg, and breast mix together without skin) were weighted aseptically and transferred to stomacher bags, followed by addition of 225 ml of 0.1% sterile peptone water. The stomacher bag was put in the Stomacher and massaged for 2 minutes to dislodge microorganisms into the solution. Then, 10 ml of the solution was taken from the filter bag and transferred to 90 ml of 0.1% sterile peptone water. Serial dilutions of 1:1000, and 1:10000 were prepared. A 1 ml of each dilution was distributed by sterile hook on plate count agar. The aerobic total count plates were incubated at 32°C for 48 hours. Psychrotrophic count plates were incubated at 7°C for 10 days. The numbers of colonies in both aerobic total count and psychrotrophic count plates were recorded. This experiment was repeated three times. Each experiment consisted of 36 whole chicken; 9 samples as control, 9 sample treated with 10% TSP, 9 sample treated with 0.5% AA, and 9 samples treated with 1% LA solutions.

Catalase activity

Catalase test was made by dipping a capillary tube into 3% hydrogen peroxide to touch the colony. Observing the bubble on top of the colony indicates a positive reaction. All colonies on plates surface were examined for catalase activity and counted the number of positive divided by the total number examined.

Off-odor

Whole chicken carcasses were stored in a refrigerator at 4°C±0.5°C, and the off-odor was evaluated at 0, 1, 3, 5, 7, 9, 11, 13, 15 day. Five panelists were trained in the smelling test (off-odor). All panelists evaluated whole chicken carcasses control and treated chicken meat at day 0, 1, 3, 5, 7, 9, 11, 13, 15 days. The chicken meat off-odor was scored by a scale. Initially, the chicken meat was scored 1, which meant no evidence of spoilage. Signs of early undesirable chicken meat were scored 2, which indicated slight off-odor. Chicken odor which was scored 3 indicated moderate off-odor. The sensory evaluation was repeated three times at 0, 1, 3, 5, 7, 9, 11, 13, 15 day. Each day, 4 whole chicken

(control sample, sample treated with 10% TSP, sample treated with 0.5% AA, and sample treated with 1% LA solutions). This experiment was repeated three times. Table 1. shows the types of test done on whole chicken meat un treated and treated.

Statistical analysis

The experiments were replicated three times (n=3). Mean value ±standard deviation was reported. Data were analyzed using analysis of variance. The probability was performed at a level of $p<0.05$ to evaluate the significance between mean value. The main value and standard deviation were used to make comparison between the TSP, LA, and AA**RESULTS AND DISCUSSION**

Evolution of aerobic and psychrotrophic counts for chicken meat (control) and samples dipped in 10% TSP, 0.5% AA and 1% LA solutions over 15 days of storage at 4± 0.5oC.

In the beginning, the initial load of aerobic total count and psychrotrophic count in control sample was 3.20.2± log CFU / g and 3.80.17± log CFU / g, respectively. Ismail *et al*. (2000) found the total viable count for various raw and processed chicken products to be in the range of 3.32–5.7 log CFU/g which is in line with what was observed in this study. A decrease in aerobic total count of chicken meats after treatments by TSP, AA, and LA solutions was observed on day 0 by 0.9, 1.0, and 0.4 log CFU / g, respectively. However, on day 7 the inhibition effectiveness of TSP, AA and LA was significantly different between these treatments and control sample. After 15 days of storage, the samples treated with TSP, AA, and LA had significantly lower ($P<0.05$) total aerobic count than that of the

Table 1. Summery the types of test done on whole chicken meat.

Time (Day)		0	1	3	5	7	9	11	13	15
Control	Type of test	TC	TC	TC	TC	TC	TC	TC	TC	TC
		PSY	PSY	PSY	PSY	PSY	PSY	PSY	PSY	PSY
		CA	CA	CA	CA	CA	CA	CA	CA	CA
		Odor	Odor	Odor	Odor	Odor	Odor	Odor	Odor	Odor
10% TSP	Type of test	TC	TC	TC	TC	TC	TC	TC	TC	TC
		PSY	PSY	PSY	PSY	PSY	PSY	PSY	PSY	PSY
		CA	CA	CA	CA	CA	CA	CA	CA	CA
		Odor	Odor	Odor	Odor	Odor	Odor	Odor	Odor	Odor
1% LA	Type of test	TC	TC	TC	TC	TC	TC	TC	TC	TC
		PSY	PSY	PSY	PSY	PSY	PSY	PSY	PSY	PSY
		CA	CA	CA	CA	CA	CA	CA	CA	CA
		Odor	Odor	Odor	Odor	Odor	Odor	Odor	Odor	Odor
0.5% AA	Type of test	TC	TC	TC	TC	TC	TC	TC	TC	TC
		PSY	PSY	PSY	PSY	PSY	PSY	PSY	PSY	PSY
		CA	CA	CA	CA	CA	CA	CA	CA	CA
		Odor	Odor	Odor	Odor	Odor	Odor	Odor	Odor	Odor

List of Abbreviation

TSP: 10% trisodium phosphate **LA:** 0.5% acetic acid **AA:** 1% lactic acid **TC:** Total Count **PSY:** psychrotrophic count **CA:** Catalase Activity

control sample. An acceptability limit of 6.3 log CFU/g of total aerobic count for fresh poultry meat was defined by ICMSF (1986). On the basis of such limit, one can conclude, that control samples were acceptable at 5 days of storage and not acceptable at 7 days, while TSP and AA treated chicken meat exceeded the acceptability limit only at the end of the storage (i.e. on day 15). LA exceeded the acceptability limit at day 13. Therefore, the shelf life of control chicken meat, defined on the basis of total aerobic count, could be extended from 5 days to 13 days when treated by TSP and AA. Similar to the present study, Sallam and Samejima, (2004) also demonstrated that the shelf life of chicken breast could be extended up to 12 days when it dipped in solution of 10% TSP and stored at 2oC. The delayed growth of aerobic spoilage microorganisms in chicken meat treated with 10% TSP, 1% LA and 0.5% AA solutions may be due to the reduction of microbial load, as well as due to the growth inhibition of aerobic bacteria as a result of an extension of the lag phase of growth, and a decrease in the growth rate during the logarithmic phase. Figure 1. shows the growth of microbial in chicken meat treated with TSP, LA, and AA refrigerated storage.

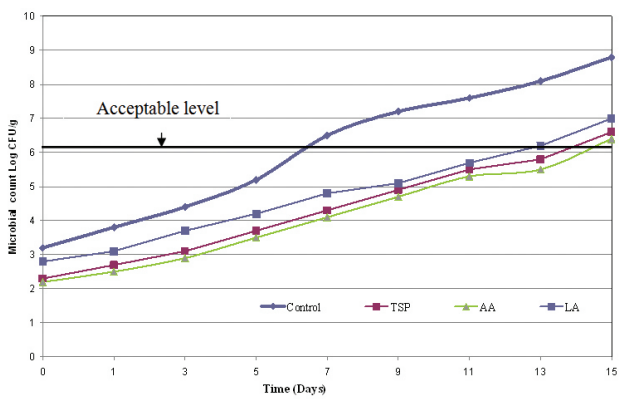

Fig.1. The growth of microorganisms in chicken meat treated with TSP, AA and LA refrigerated storage.

Figure 2 shows the evolution of psychrotropic count in chicken meat stored at 4oC±0.5 for 15 days. Psychrotrophic count of chicken meat increased during storage at 4oC±0.5 for 15 days. However, psychrotrophic count in treatment samples with TSP, AA and LA was lower than control sample. Towards the end of the storage period, the inhibition effectiveness of TSP and AA was greater than LA. Comparing between theses results and acceptable limit designed by ICMSF (1986), the shelf-life periods of samples treated with TSP, AA and LA were 13, 13, and 11 days, respectively. On these days the psychrotrophic count was lower than acceptable limit of 6.3 log CFU/g.

In the beginning, psychrotrophic count was 3.80.17± log CFU/ g in the case of control samples. After 5 days of storage at 4oC, plate count agar was log 5.20.2± and 6.00.3± for psychrotrophic count. The results show that psychrotrophic counts were significantly ($p<0.05$) higher than plate count agar throughout the storage period at 4oC±0.5. Psychrotrophic bacteria are capable of surviving and developing or even thriving in a cold environment over a wide temperature range and they can grow at temperatures close to or below freezing (Hébraud and Potier, 1999). Microbial spoilage of refrigerated chicken meat is primarily due to the presence of psychrotrophic bacteria. Psychrotrophic bacteria can grow on carcass in the chill during chilling period (Jay, 1992). Most bacteria contributing the aerobic plate count agar were psychrotrophic bacteria since the amount of the aerobic bacteria grown at 4°C±0.5 was nearly identical to total aerobic bacteria. The use of TSP, AA, and LA as shown in the present study, resulted in an extension of shelf-life of raw chicken meat by 8, 8 and 6 days, respectively.

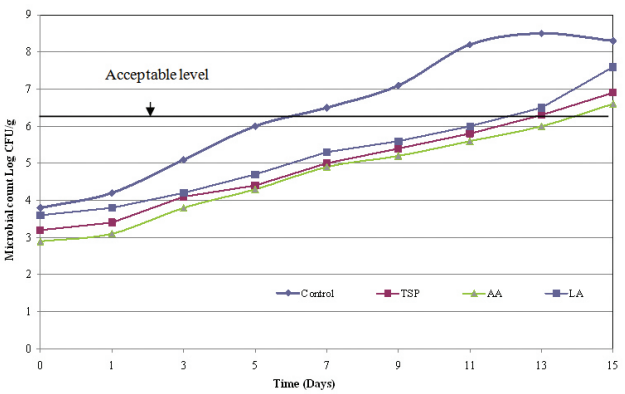

Fig.2. The growth of microorganisms in chicken meat treated with TSP, AA and LA refrigerated storage.

Comparison between TSP, AA, and LA

Figure 3. shows the amount of reduction of microbial count in chicken meat treated by TSP, AA, and LA. On day 0 the reduction of microbial count by TSP, AA, and LA was 0.90.1±1 ,0.1±, and 0.40.03± log CFU/ g, respectively. The reduction of microbial count in chicken meat treated by TSP, AA, and LA increased during storage time. On day 7 the reduction of microbial count by TSP, AA, and LA was 2.20.26±2.4 ,0.2±, and 1.70.18± log CFU/ g, respectively. This result indicated that the AA was higher reduction than and TSP and LA, and TSP was higher reduction than LA. However, there was no significant different between AA and TSP.

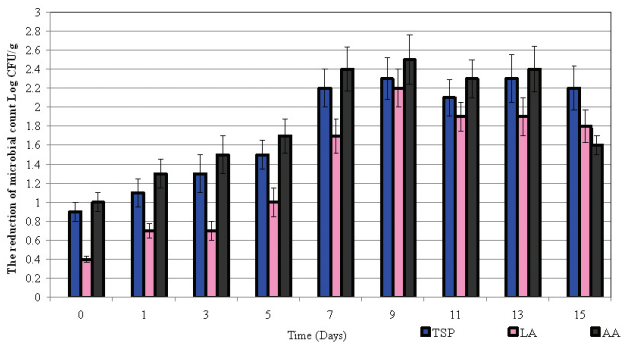

Fig.3. The comparison of reduction of microbial count in chicken meat treated with TSP, AA and LA refrigerated storage.

Development of off-odor in chicken meat

Figure 4 shows the development of off-odor in samples untreated and treated with TSP, LA, and AA chicken stored at 4oC for 15 days. The sensory quality was not adversely affected by LA, AA, and TSP. The odor was observed in control samples at day 7, LA at day 13 and TSP and AA at day 15 storage at 4°C. Off-odor in chicken meat dipped in 10% TSP, 0.5% AA and 1% LA were rated significantly lower than the control at day 7 and 9 and 11. These results indicated that TSP and AA solutions have good potential as dips to sanitize chickens carcasses.

Acceptability as a composite of off-odor was estimated using a descriptive scale ranging from 1–3, where: 1= no odor, 2 = slight odor and 3= extreme odors. The chicken meat was considered unacceptable as soon as the mean score exceeded 1. A strong correlation was observed between the development of the characteristic spoilage off-odor and the number of psychrotrophic count; off odor was first observed when psychrotrophic count reached 6.5 log CFU/ g. More offensive odor was observed when psychrotrophic count reached 8 log CFU/ g. It has been demonstrated that Pseudomonas spp. which are found in large numbers in spoilage chicken meat, are capable of growing at -3oC (Patsias *et al*., 2006). They produce more offensive odors as compared to the typical spoilage microorganism in chicken meat, It is assumed, therefore, that their growth was responsible for the intensive off-odor at the end of the storage period.

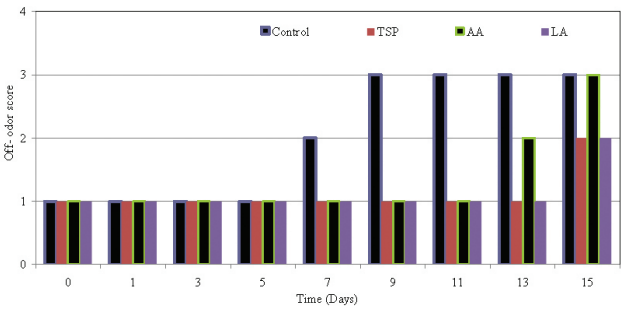

Fig.4. The development of Off-odor in chicken meat treated with TSP, AA and LA refrigerated storage.

The development of catalase activity by testing the colonies on plate

The present study focused on the monitoring of the numbers of catalase negative and positive bacteria on plates. Table 2. shows the development of a number of catalase positive colonies in chicken meat stored at 4°C for 15 days. This experiment was repeated three times. On day 0 the percentage of catalase positive microorganism in the control samples was 78%. On day 9, catalase activity reached 94% and after that decreased to 80% on the day 15. The percentage of catalase activity in treated samples with TSP, AA, and LA increased on day 0 from 70%, 70% and 73% to 92%, 94% and 94% on day 9, respectively. Increased catalase activity was due to the predominant psychrotropic bacteria associated with the spoilage of the refrigerated chicken meat (Doyle *et al*, 1997). Most of psychrotropic bacteria are Gram negative and catalase positive. On the end day the catalase activity decreased in treated sample with TSP, AA, and LA to 86%, 88% and 85%. The decrease in the catalase activity at the end of the storage was

Table 2. Shows the number of colonies catalase positive in chicken meat stored at 4oC for 15 days.

	Control			LA			AA			TSP		
Time Day	# of colonies on plate	# of CAT POS	% Of CAT ACT	# of colonies on plate	# of CAT POS	% Of CAT ACT	# of colonies on plate	# of CAT POS	% Of CAT ACT	# of colonies on plate	# of CAT POS	% Of CAT ACT
0	158±7	123±4	78%	63±4	46±3	73%	158±6	111±4	70%	200±6	140±5	70%
1	63±4	51±3	81%	125±8	100±6	80%	31±4	24±6	77%	50±4	38±4	76%
3	44±4	38±6	86%	50±4	42±5	84%	79±5	65±6	82%	125±8	101±7	81%
5	25±3	23±5	90%	158±9	140±6	88%	32±5	28±7	87%	50±7	43±6	86%
7	60±5	56±4	93%	63±5	57±4	90%	125±6	115±4	92%	200±4	180±3	90%
9	100±9	94±7	94%	125±6	118±5	94%	50±4	47±5	94%	80±6	74±5	92%
11	158±7	142±4	90%	50±4	47±5	94%	199±5	187±4	94%	32±3	30±4	93%
13	32±4	29±6	92%	158±7	148±4	93%	32±4	29±6	91%	158±8	145±7	92%
15	63±5	51±5	80%	100±6	85±6	85%	160±6	141±7	88%	250±5	215±6	86%

Abbreviation: **CAT :** Catalase **ACT:** Activity **POS:** Positive

This experiment was repeated three times (n=3)

due to the increase in the catalase content during the logarithmic growth phase which reached a maximum just before the stationary growth phase, and then declined exponentially (McCarthy and Hinshelwood, 1959).

CONCLUSION

Interestingly, in the present study, low number of aerobic total count and psychrotrophic bacteria were observed in chicken meat treated with TSP, AA, and LA, noted throughout the entire storage period, which may be due to the inherent sensitivity of Gram-negative bacteria to extrinsic factors such as pH. The use of TSP, AA, and LA as shown in the present study, resulted in an extension of shelf-life of raw chicken meat by 8, 8, and 6 days, respectively. The shelf life of chicken meat (control) was, 5 days at refrigerated temperatures 4±0.5°C. The total plate count reached 6.0 log CFU/g normal shelf life is 5 days. As results extending the shelf life by 6 or 8 days, can provide economic benefit.

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