EFFECT OF CITRIC ACID, VINEGAR AND SODIUM LACTATE ON STAPHYLOCOCCUS AUREUS EXPERIMENTALLY INOCULATED IN FISH FILLET.

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ABSTRACT

This study was designed to evaluate the effect of sodium lactate, citric acid and vinegar on Staphylococcus aureus (S. aureus) in fish fillet. Thirty five fish fillet samples were divided into seven group including the control and inoculated with 1ml containing 105 cfu S. aureus culture, then subjected to six treatments: sodium lactate 2 &3%, citric acid 1 &2% and vinegar 3 &5% , all samples and control were stored at 4°C and followed up at 0, 24 and 48hr. for counting S. aureus. Statistical analysis of the result revealed that, the count of S. aureus was decreased in all treated samples. The highest reduction percentage of S. aureus count was in samples treated with vinegar 5% after 48hr. (97.63%) followed by citric acid (95.21%) then, sodium lactate (82.79%). Therefore vinegar can be used to improve the biological quality of fish fillet during short storage at refrigerator temperature specially for reducing initial S. aureus contamination.

INTRODUCTION

Foods are not only of nutritional value but also considered as an ideal culture media for microbial growth. Like meat, fish and other sea foods may be spoiled by autolysis, oxidation and fermentation bacteria or most commonly by combination of the aforementioned causes. Most fish

flesh, is considered to be more perishable than meat because it is easy and more rapidly undergoing autolysis by the fish enzymes, and because of the less acid reaction of fish flesh that favors microbial growth ***(Jay, 1978).***

Except for sea foods, the food industry relies heavily upon the use of antimicrobial agents to extend the shelf life and preserve freshness of product. The problem for the food industry is fulfilling the demands of minimum changes in food quality and maximum security, ***(Barakat et al., 2006).*** Foods that require handling during preparation and kept at slightly elevated temperatures after preparation are frequently involved in Staphylococcal food poisoning. *Staphylococcus aureus* are fairly heat sensitive, however, heat cannot be relied on to make food safe as the toxin is heat stable. ***(Hocking, 2003).***

The organic acids and their salts are beneficial for refrigerated meat, poultry and fish product by inhibiting spoilage, microbial growth and pathogens, ***(Sallam, 2007a, b and sallam and Samjima, 2004).*** Extensive researches have been carried out to understand and characterize the inhibitory actions of various organic acids on growth of microorganisms.

Organic acids have been used to control microbial growth, improve sensory attributes and extend the shelf life of various food systems including fish, ***(Boskou and Debevere, 2000).*** The use of any anti microbial depends on several factors, such as desired effect, legal limits of use and effect on food. The effectiveness of organic acids as antimicrobials differ widely based on concentration, pH, initial bacterial load and the concentration of the non dissociated form, ***(Beth et al.,***

2004). The non dissociated acid caused more than 50% growth inhibition of *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* at pH levels above 6, ***(Eklund, 1983).*** Acetic acid commonly called vinegar is a mono carboxylic acid with a pungent odor and taste ***(Pundir and Jain, 2011).*** In addition compounds such as ascorbic and citric acids are used countering oxidative rancidity. In addition sodium lactate has been evaluated for anti-oxidative effects ***(Lamkey et al., 1991).***

In spite of the fact that, sodium lactate has a high safety margin, its' usage in food industry still very limited (up to 4%). Sodium lactate addition can reduce the product water activity, there by preventing the growth of microorganisms, ***(Shweb, 2009).*** Sodium lactate has a mild saline taste and is commonly used to extend shelf life and increase food safety as it has abroad antimicrobial action, against yeast and fungi, and is effective at inhibiting most spoilage and pathogenic bacteria ***(Silberberg, 2009).***

The present study was conducted to evaluate the effect of sodium lactate, citric acid and vinegar against *staphylococcus aureus* on fish fillet samples.

MATERIAL AND METHODS

Samples: Thirty five fish fillet samples (each was 250-300 gm in weight) were purchased from retail market on the same day of the experiment.

Bacterial strain: *Staphylococcus aureus* cultures ATCÇ 6538P was obtained from Animal Health Research Institute.

Treatments used: Sodium lactate solution (2&3%), Citric acid solution (1&2%) and Vinegar (acetic acid) solution (3&5%) were used for treatment of samples.

Preparation of bacterial strain: According to **Pantarika et al., (2009),** *Staphylococcus aureus* culture was sub cultured in tryptic soy broth (Merck) at 37C° for 24 hr prior to use in the experiment. The active cultures were diluted in 0.1% sterile peptone water and counted to ensure the initial counts of 10^5 Cfu/ml on all tested sampies. Each sample was subjected to UV light at 253.7nm in the biological safety cabinet (Class II -UK) to be free from *S. aureus* and other organisms.

Experimental design:

Fish fillet samples were divided into seven groups including the control. Prior to addition of treatment, each sample within the groups was inoculated with 1ml of the prepared culture strain, then samples were subjected to six treatments as follow: sodium lactate 2 &3%, citric acid 1&2% and vinegar 3 &5% ,the last group was kept as control without any treatment. The treatments were applied by immersion of the fish fillet samples in the treatments solutions for 5 minutes and drained on the sterile screen for 10 minutes. All sample groups including the control were stored at 4°C; five replicates from each group were examined for counting of *S. aureus* at 0, 24 and 48hr.according to **(Iso 6888, 1999).** The microbial results of five replicates from each group of fish fillet samples were statistical analyzed.

Statistical analysis:

Minimum, maximum, mean and standard error of mean was used to describe data. One-Way ANOVA test was used to compare the reduction effects of different treatments as compared to control group on *S. aureus* counts on fish fillet samples. *P* value was considered significant if less than 0.05. These tests were analyzed using the Statistical Package for Social Scientists (SPSS) for windows 16.0 (SPSS Inc., Chicago, IL, and USA).

RESULTS

Table (1): Statistical analytical results of the effects of sodium lactate 2and 3% on growth of *Staphylococcus aureus* inoculated in fish fillet. (n=5)

	Sodium lactate 2%			Sodium lactate 3%			Control		
	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
Min.	7.5×10^4	5.0×10^4	3.1×10^4	6.0×10^4	5.1×10^4	1.0×10^4	1.0×10^5	9.5×10^4	9.0×10^4
Max.	8.0×10^4	6.5×10^4	4.0×10^4	6.9×10^4	5.7×10^4	2.3×10^4	1.0×10^5	9.9×10^4	9.6×10^4
Mean	7.7×10^4	6.1×10^4	3.6×10^4	6.4×10^4	5.5×10^4	1.6×10^4	1.0×10^5	9.7×10^4	9.3×10^4
S.E.	1.5×10^2	5.4×10^2	4.5×10^2	3.5×10^2	2.8×10^2	8.2×10^2	0.00	2.3×10^2	3.2×10^2

Min. = Minimum Max. = Maximum S.E. = Standard Error

Table (2): Statistical analytical results of the effects of citric acid 1 and 2 % on growth of *Staphylococcus aureus* inoculated in fish fillet. (n=5)

	Citric acid 1%			Citric acid 2%			Control		
	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
Min.	6.1×10^4	4.7×10^4	1.0×10^4	4.2×10^4	3.3×10^4	4.1×10^3	1.0×10^5	9.5×10^4	9.0×10^4
Max.	6.7×10^4	5.4×10^4	4.3×10^4	4.7×10^4	3.8×10^4	4.8×10^3	1.0×10^5	9.9×10^4	9.6×10^4
Mean	6.4×10^4	5.0×10^4	2.7×10^4	4.4×10^4	3.6×10^4	4.5×10^3	1.0×10^5	9.7×10^4	9.3×10^4
S.E.	2.4×10^2	3.0×10^2	1.5×10^3	2.7×10^2	2.2×10^2	3.2×10	0.00	2.3×10^2	3.2×10^2

Min. = Minimum Max. = Maximum S.E. = Standard Error

Table (3): Statistical analytical results of the effects of vinegar 3 and 5 % on growth of *Staphylococcus aureus* inoculated in fish fillet. (n=5)

	vinegar 3%			vinegar 5 %			Control		
	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
Min.	4.6×10^4	3.4×10^4	3.0×10^3	3.0×10^4	1.9×10^4	1.7×10^3	1.0×10^5	9.5×10^4	9.0×10^4
Max.	5.3×10^4	4.1×10^4	1.0×10^4	3.9×10^4	2.7×10^4	1.0×10^4	1.0×10^5	9.9×10^4	9.6×10^4
Mean	5.0×10^4	3.7×10^4	7.3×10^3	3.5×10^4	2.3×10^4	2.2×10^3	1.0×10^5	9.7×10^4	9.3×10^4
S.E.	3.6×10^2	3.2×10^2	3.1×10^2	4.4×10^2	4.3×10^2	2.7×10^2	0.00	2.3×10^2	3.2×10^2

Min. = Minimum Max. = Maximum S.E. = Standard Error

Table (4): Effect of Sodium lactate 2 and 3 % on reduction of *Staphylococcus aureus* count during cold storage period (0-48hr).

	Sodium lactate 2%			Sodium lactate 3%		
	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
Mean	7.7×10^4	6.1×10^4	3.6×10^4	6.4×10^4	5.5×10^4	1.6×10^4
Control	1.0×10^5	9.7×10^4	9.3×10^4	1.0×10^5	9.7×10^4	9.3×10^4
Reduction %	23.00	37.11**	61.29**	36.00	43.30**	82.79**

** highly significant

Table (5): Effect of citric acid 1 and 2 % on reduction of *Staphylococcus aureus* count during cold storage period (0-48hr).

	Sodium lactate 2%			Sodium lactate 3%		
	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
Mean	6.4×10^4	5.0×10^4	2.7×10^4	4.4×10^4	3.6×10^4	4.5×10^3
Control	1.0×10^5	9.7×10^4	9.3×10^4	1.0×10^5	9.7×10^4	9.3×10^4
Reduction %	36.00	48.45**	70.96**	56.00	62.88**	95.21**

** highly significant

Table (6): Effect of vinegar 3 and 5 % on reduction of *Staphylococcus aureus* count during cold storage period (0-48hr).

	vinegar 3%			vinegar 5 %		
	0 hr	**24 hr**	**48 hr**	**0 hr**	**24 hr**	**48 hr**
Mean	5.0×10^4	3.7×10^4	7.3×10^3	3.5×10^4	2.3×10^4	2.2×10^3
Control	1.0×10^5	9.7×10^4	9.3×10^4	1.0×10^5	9.7×10^4	9.3×10^4
Reduction %	50.00	61.85**	92.15**	65.00	76.29**	97.63**

**highly significant

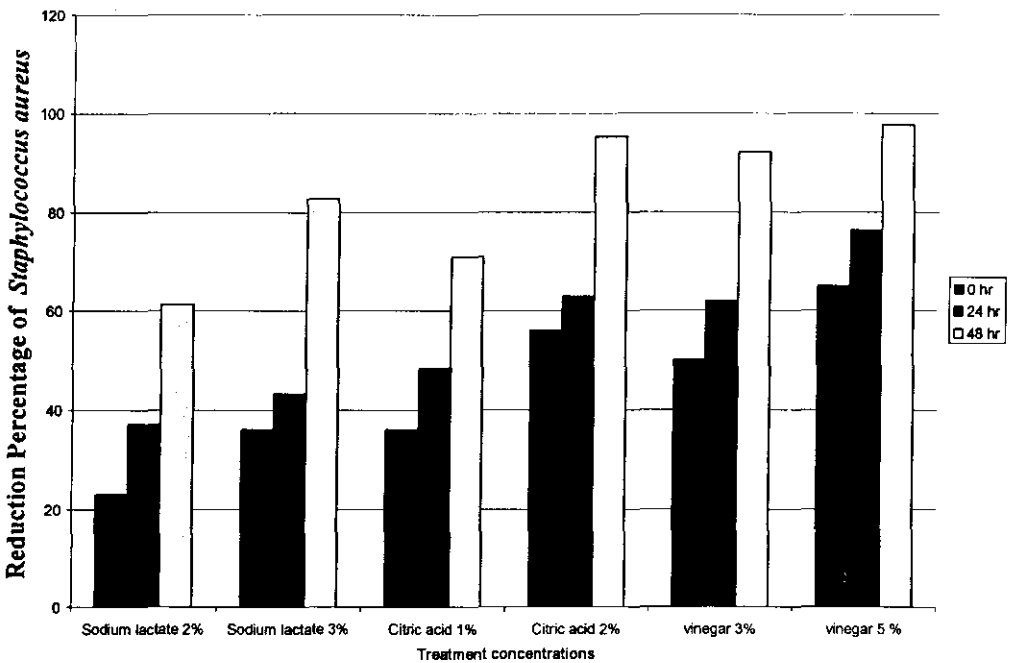

Fig. (1): Effect of different treatment on reduction of *Staphylococcus aureus* count during cold storage period (0-48hr).

DISCUSSION

Fish fillet samples, were artificially contaminated with *S. aureus* and treated with different concentration of sodium lactate, citric acid and vinegar before being stored at 4°C.The results tabulated in table (1) revealed that the mean values of *S.aureus* counts at 0, 24 and 48 hr. of sodium lactate 2% treatment were 7.7 X 10^4 ±1.5 X 10^2, 6.1 X 10^4 ± 5.4 X $10^2$and 3.6X10^4 ±4.5 X 10^2 respectively, while the mean values with sodium lactate 3%were 6.4 X 10^4±3.5 X 10^2, 5.5 X 10^4±2.8 X 10^2 and 1.6 X10^4 ±8.2 X 10^2 respectively. The present results indicated that, increase of concentration of sodium lactate and storage period play a role in growth inhibition of *S.aureus* , similar findings were recorded by ***(Drostinos et al., 2006)*** who stated that The microbial inhibition increased with increasing concentration of sodium lactate. In this concern ***Wit and Rombouts, (1990)*** demonstrated in their study that, sodium lactate antimicrobial effect towards *Staphylococcus aureus*, is particularly evident from increasing lag phase, decreasing growth yield, and somewhat less from decreasing growth rate.

Regarding to Table (2) the statistical analysis of *S. aureus* counts in samples treated with citric acid showed that, the mean values at 0, 24 and 48 hr. with citric acid1% treatment were 6.4X 10^4±2.4 X 10^2, 5.0 X 10^4±3.0 X 10^2 and 2.7 X 10^4±1.5 X 10^3 respectively. On the other hand, the mean values of citric acid 2% were 4.4 X 10^4±2.7 X 10^2, 3.6 X 10^4 ±2.2 X 10^2 and 4.5 X 10^3±3.2 X10 respectively. In this respect the antibacterial activity of citric acid dependent on pH, concentration and anion effects ***(Young and Foegeding, 1993)***.

The results in table (3) showed that, the mean values of *S.aureus* count at 0,24and48hr. were 5.0 X 10^4 ±3.6 X 10^2, 3.7 X 10^3±3.2 X 10^2 and 7.3 X 10^4 ±3.1 X 10^2respectively in case of treatment with 3% vinegar. While the mean values were 3.5 X 10^4 ±4.4 X 10^2, 2.3 X 10^4±4.3 X10^2 and 2.2X10^3 ±2.7 X 10^2 respectively at 0, 24 and 48 hr. in case of treatment with vinegar 5%. Vinegar has shown to reduce *S. aureus* in fish fillet samples in both concentrations after 24 and 48 hr. of cold storage. Such results are in agreement with those reported by ***Pantarika et al., (2009).*** Vinegar has antimicrobial capabilities due to its ability to lower the pH and result in instability of bacterial cell membranes ***(Jay, 1992).***

From the results achieved in tables 1, 2, 3 we can notice that, the highest count of *S.aureus* was observed in untreated control samples which show very slight and slowly decrease in number. This may be due to the effect of low storage temperature at 4°C and possible presence of competitive psychotrophic microorganisms. The results agreed with the other work recorded by ***Smulders and Greer, (1998) and Dubal et al., (2004).*** In addition, ***Drostinos et al., (2006)*** reported that microbial inhibition increased with increasing concentration of organic acid and their salts.

Tables (4, 5, 6) and Fig. (1) showed the reduction of *S.aureus* count in various treatments as compared to control, according to statistical analytical results, there were significant reductions (P<0.05) in *S. aureus* counts between treated and control group during storage with interaction of acid treatment and storage period mean ,while no significant reduction in control group. The reduction percent at 0, 24 and 48 hr. in samples

treated with 2% sodium lactate was 23.00%, 37.11% and 61.29% and it was 36.00%, 43.30% and 82.79% respectively in treatment with 3% sodium lactate in (Table 4 & Fig. 1). The reduction effects of citric acid 1% as shown in Table (5) & Fig.(1) at 0, 24 and 48 hr were 36.00%, 48.45% and 70. 96% while that of 2% citric acid concentration were 56.00%, 62.88% and 95.21% respectively. On the other hand, the reduction effect of vinegar at 0, 24 and 48 hr were 50.00%, 61.85% and 92.15% for 3% concentration and were 65.00%, 76.29% and 97.63% respectively for 5% concentration of vinegar (Table 6 & Fig. 1).

From the present results we can noticed that, the reduction of *S.aureus* counts in treated samples with acids were more than sodium lactate, the fact that the anti microbial effect may be caused by pH reduction below the growth range and metabolic inhibition by the undissociated molecules ***(Dubal et al.,2004).*** On the other hand the highest reduction of *S.aureus* count was obtained after 48hr by 5% vinegar treatment (97.63%) citric acid2% treatment, (95.21%) and vinegar 3% treatment (92.15%) than citric acid 1%and sodium lactate. ***Min-suk et al., (2003)*** have pointed out that, the effectiveness of acetic acid in retarding growth or killing food-borne pathogenic bacteria may vary among treatments depending on the percentage of un-dissociated acid at a given pH. In addition ***Jamilah, et al., (2008)*** reported that, acetic acid is generally regarded as safe as for miscellaneous and general-purpose usage.

Therefore, it can be concluded that, vinegar was more efficient and had antimicrobial effects superior to citric acid and sodium lactate, so, it can be used to improve the safety of fish fillets during short storage.

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تأثير حمض الستريك والخل و الصوديوم لاكتيت على الميكروب العنقودي الذهبي المحقون في شرائح الأسماك.

عزة على حسين التابعي و أماني محمود شلبي

أجريت هذه الدراسة لاستبيان تأثير كل من الصوديوم لاكتيت ،حمض الستريك و الخل على الميكروب المكور العنقود الذهبي في شرائح الأسماك.وقد تم تقسيم 35 عينة على سبعة مجموعات متضمنة المجموعة الضابطة وتم حقنهم جميعا بمقدار 1ملى من تركيز 510 للميكروب وبعد ذلك تم إضافة كل من الصوديوم لاكتيت بتركيز 2&3٪ وحمض الستريك بتركيز 1&2٪ والخل بتركيز 3&5٪ كل على حدة.وقد تم حفظ كل العينات متضمنة المجموعة الضابطة عند درجت 4 درجة مئوية ومتابعتها عند صفر و24 و48 ساعة من الحقن.هذا وقد أسفرت النتائج الاحصائية عن حدوث تناقص في العد الكلى للميكروب المكور العنقودي الذهبي في كل من العينات المعالجة سواء كانت معالجه با لصوديوم لاكتيت ، حمض الستريك أو بالخل، ألا انه قد لوحظ أن أعلى نسبه لاختزال الميكروب كانت في العينات المعالجة بالخل خاصة(97.63٪) تلك ذات التركيز 5٪ بعد 48 ساعة من الحقن ، يليها العينات المعالجة بحمض الستريك (95.21٪) ثم تلك المعالجة بالصوديوم لاكتيت (82.79 ٪). وعلى ذلك فانه يمكن تحسين الحالة الصحية لشرائح الأسماك المحفوظة لوقت قصير في الثلاجة وذلك لتقليل تواجد ميكروب المكور العنقودي الذهبى.