



Efficacy of Acidified sodium chlorite in controlling *Listeria monocytogenes* in salmon fillet

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ABSTRACT

The purpose of this study was planned out to evaluate the effectiveness of Acidified Sodium Chlorite (ASC) on the viability of *L. monocytogenes* by contaminating Salmon fish samples with 1×10^8 cfu/g of *L. monocytogenes*, followed by dipping the contaminated samples in different concentrations of ASC 250, 500 and 1000 ppm. The samples were stored in refrigerator at 4°C to be examined for *L. monocytogenes* count at Zero day, First, third, fifth and seventh day of refrigeration storage. The results revealed that the counts of *L. monocytogenes* in salmon fish samples dipped in different ASC concentrations of 250, 500 and 1000 ppm in zero (treatment day), were 1×10^7 , 3.8×10^6 and 1×10^6 cfu/g respectively, and in the first day after storage the counts were 8×10^6 , 1×10^6 and 1×10^5 cfu/g respectively while in the third, fifth and seventh day the counts were reduced to be <10 cfu/g.

Keywords: ASC, Salmon fillet, *L. monocytogenes*

(<http://www.bvmj.bu.edu.eg>)

(BVMJ-28(1): 186-190, 2015)

1. INTRODUCTION

Changes in food production and demands of growing society have increased the number of incidences of food borne illness. The microbial pathogens in food are the cause of 6.5 million cases of human illness and up to 9.000 deaths in the U.S. each year (Buzhy, 2002). *Listeria monocytogenes* has been labeled as a leading of death due to food borne illness (Buzhy, 2002). *L. monocytogenes* has also developed as a food borne pathogen due to several other factors; one of such causes is the development of technology, which provides cold storage give adequate temperature for the growth and survival of *L. monocytogenes* (Richelle Lynn Beverly, 2004). The main concern of the food industry is that *L. monocytigenes* can grow at refrigerator temperature and is resistant to various environmental conditions (Tompkin, 2002). Sea food products might

create potentiality cross contamination with *L. monocytogenes*. Therefore, efforts to minimize cross contaminated products are needed. The food agriculture organization reported that the cold smoked fish (Salmon fillets) have been found to be contemned with *L. monocytogenes* at 100cfu/g and the temperature applied during smoking process is not sufficient to inactivate all *L. monocytogenes* cells in fish products (Tsui-Yin Wong, 2009). The purpose of antimicrobial agents is to reduce or eliminate microbial activity. The use of antimicrobial agents allows products to be microbial safe with the enhanced ability to extend shelf life. Consuming healthier foods is a demanding request for protection of public health and safety. Acidified sodium chlorite (ASC) is a product of the acidification of sodium chlorite (NaClO_2) using citric acid, it works by forming stable Oxychlorus antimicrobial intermediates

(chlorite, chlorate and chlorine dioxide) as it comes into contact with organic matter (Gordon et al., 1972, Kross, 1984). ASC is applied onto the surfaces of the food at low levels. The Oxychlorine compounds are stable, and found in acidified sodium chlorite treatment solution, forming chlorite, chlorate and chlorine dioxide after mixing and after contact with food surfaces. Chlorine dioxide that forms is either evaporated or reduced without residual traces. (Food Standards Australia, New Zealand, 2003). Mixture of sodium Chlorite and citric acid forms (acidified sodium chlorite) which is applied onto food surfaces to reduce the number of microorganisms. The mix is applied by spraying or dipping. The use of sodium chlorite is technologically justified science it has a function in food as an antimicrobial agent, and there are no significant public health and safety concerns associated with its use as it has no toxicological concerns so encouraging an efficient and internationally competitive food industry (Food Standards Australia, New Zealand, 2003). The use of acidified sodium chlorite is intended as an antimicrobial intervention on a variety of raw food types to reduce the microbial contamination arising from the presence of pathogenic and spoilage species of microorganism.

2. Material and methods

2.1. Bacteriological strain :

The bacterial strain used was *Listeria Monocytogenes* (ATCC 35152) which obtained from the reference strain bank of Food Hygiene Department, (AHRI).

2.2. Inoculum preparation:

The strain was deep-frozen stored in a cryo protective vial containing preservative solution at -70°C , Cryobead (inoculums) of the strain was cultivated in tryptic soy broth over night at 35°C . Then cells were

centrifuged for 10 min at 8000rpm. Supernatant was discarded and cells were washed three times and re-suspended in sterile 0.1% peptone water. The cells were diluted in peptone water adjusted to obtain the desired inoculums level (10^7 cfu/ml) (Osman, 2008).

2.3. Preparation of Acidified Sodium Chlorite:

Acidified sodium chlorite solution (ASC) was prepared by mixing 0.5g/lof sodium chlorite with 1 g/l citric acid and the solution PH was 2.62 then allowed to activate for 10 minute. Then diluted with sterile distilled water to form different dilutions of 250, 500 and 1000 (ppm) with different PH (3.25, 3.22 and 3.15), the various treatments were placed in sterile containers and freshly prepared just prior examination (Inatsu, et. al., 2010). $\text{NaClO}_2 + \text{H}(\text{acid}) \rightarrow \text{HClO}_2$ (chlorous acid) $\rightarrow \text{CLO}$ (chlorine dioxide), which has antimicrobial effect.

2.4. Sample preparation:

Eighteen hours *L.monocytogenes* culture was decimally diluted to 10^8 cfu/ml in phosphate buffer saline (PBS) solution. The salmon fish samples was cut into cubes almost equal in size and inoculated with the prepared *L.monocytogenes* strain, then allowed to air dry for 30 minutes in laminar flow (class II safety cabinet). The samples (except control) were then dipped into prepared different concentrations of Acidified Sodium Chlorite (ASC) (250,500 and 1000 ppm) for 20 minutes then left to be air dried for 30 minutes in laminar flow and kept in sterile polyethylene bags in refrigerator at 4°C to be examined at zero, 1st, 3rd, 5th and 7th day of storage for *L.monocytogenes* viable cell count. The experiment was repeated triple (FDA, 2011).

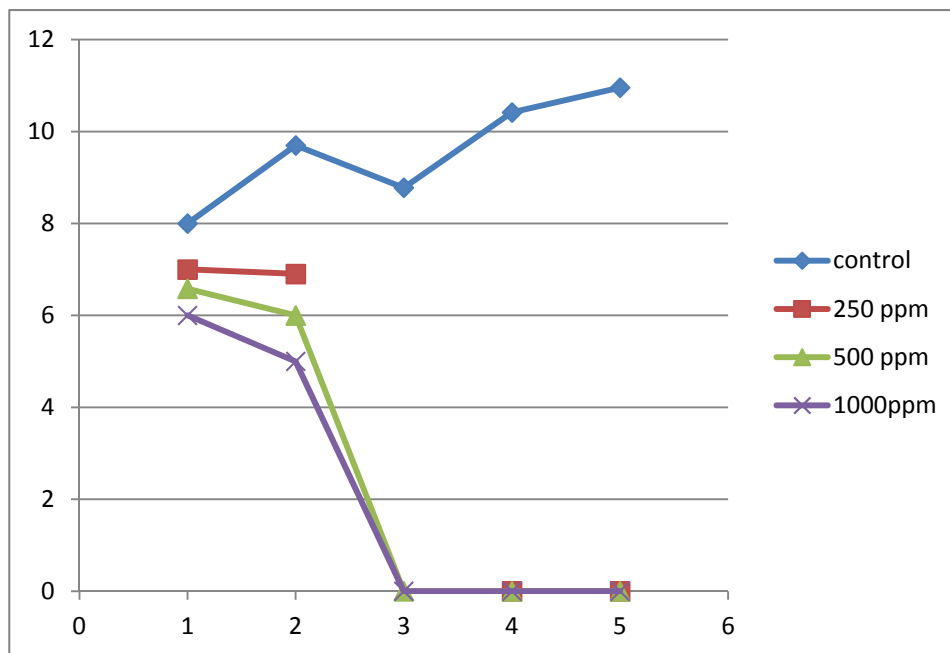
3. Results:

Table1: Effect of different concentrations of Acidified Sodium Chlorite (in ppm) on the viable count of inoculated *L. monocytogene* in salmon fish

Day	ASC Conc)	Blank fish sample Control-ve)(250 ppm	500ppm	1000ppm
Zero day (after 1hr)		1x10 ⁸ cfu/g	1x10 ⁷ cfu/g	3.8x10 ⁶ cfu/g	1x10 ⁶ cfu/g
1 st day		5x10 ⁹ cfu/g	8x10 ⁶ cfu/g	1x10 ⁶ cfu/g	1x10 ⁵ cfu/g
3 rd day		6x10 ⁹ cfu/g	<10 cfu/g	<10cfu/g	<10cfu/g
5 th day		2.6x10 ¹⁰ cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
7 th day		9x10 ¹⁰ cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g

ND: Not detectable level*

Fig 1: Effect of different concentration of ASC (ppm) on *L. monocytogenes* in salmon fillet:



4. DISCUSSION

Food borne diseases and food poisoning are common throughout the world. Fresh fish is highly perishable and microbial spoilage is one of the main causes of limited shelf life and safety as well as contamination with pathogenic microorganisms by mishandling of food. Food borne illness outbreaks are a serious problem and results some times in

significant death. One of the main pathogens commonly associated with seafood contamination is *Listeria* (Norhana *et al.*, 2010). The use of antimicrobial agents in food processing is a way to produce safer products. Acidified Sodium Chlorite (ASC) possesses antimicrobial properties and is intended for using primarily as a spray or dipping solution for fish (Madduri V. Rao, 2007). According to

table (1), the initial count of control samples In zero day was 1×10^8 cfu/g while count differed in the different dipped samples with different ASC concentrations. As it was 1×10^7 , 3.8×10^6 and 1×10^6 cfu/g in the dilutions 250,500 and 1000 ppm respectively, while *L. monocytogenes* in the control sample count after the first day of refrigeration storage was 5×10^9 cfu/g. While in samples were dipped in 250,500 and 1000 ppm ASC *L. monocytogene* counts were of 8×10^6 , 1×10^6 and 1×10^5 cfu/ respectively. The control sample showed marked increase in the third day of experiment, while it was <10 cfu/g (non detectable level) for samples dipped in ASC of the three concentrations in the third day. In the fifth and seventh day of storage *L. monocytogenes* increased to be 2.6×10^9 and 9×10^{10} cfu/g respectively, While the samples dipped in 250,500 and 1000 ppm ASC concentrations remained constant at <10 cfu/g. These results indicated that, the inoculated control fish fillet samples with *L. monocytogenes* showed obvious increase in their bacterial count during refrigeration storage, which approved that *L. monocytogenes* can stay viable and even grown well in the refrigeration temperature reaching 9×10^{10} cfu/g at the end of the experiment day(7th day) of the study (Table1, Fig 1). These results agreed with those reported by Salyrs and Whitt (2002) who illustrated that *L. monocytogenes* can multiply over a wide range of temperatures, especially at refrigeration temperature. While the results of *L. monocytogenes* count in treated Salmon fish, fillet samples with ASC gave great indication on the effect of acidified sodium chloride (ASC) as antibacterial agent. In the beginning of this study at zero day after one hour of dipping in ASC the count decreased by one log cfu /g in the 250ppm concentration and decreased by two logs cfu/g in samples dipped in 500 and 1000ppm concentration comparing to the control inoculated non treated samples. After the first day of refrigeration storage, *L. monocytogenes* count increased in the control samples by

one log cfu/g to be 5×10^9 cfu/g but the treated samples *L. monocytogenes* count with ASC were decreased by another 3 log cfu /g at 250 and 500ppm, while it decreased by four log cfu/g at 1000ppm of ASC concentration as compared with control samples count under the same refrigeration conditions. At the third day of refrigeration storage, *L. monocytogenes* count reached <10 cfu/g for all three concentrations and the same count in the fifth and seventh day of examination indicating the obvious clear inhibitory effect of ASC on the viability of *L. monocytogenes* in the examined fish fillet samples. Similar other studies evaluated the effectiveness of ASC as a processing aid for dipping or spraing to control *L. monocytogenes* aiding in the safety of ready to eat products (Beverly, 2004). Also Su and Morrissey (2003) reported a 0.43 log cfu/cm² reduction of *L. monocytogenes* on whole Salmon washed with a 50 ppm acidified sodium chlorite solution for one minute and found a 0.62 log reduction after 7 days of storage as compared with initial count of *L. monocytogenes* (10^4 cfu/g). Beverly et.al. (2004) investigated the effect of ASC against *L. monocytogenes* on the surface of cooked Roost beef with different concentrations of 250,500 and 1000 ppm ,samples had approximately 2.5 log cfu/g reduction in count.

In this study, it can be concluded ASC has antimicrobial effect on *L. monocytogenes* by dipping and appeared in the first day of storage and increased in the third day, the use of 250 and 500 ppm concentration of ASC is recommended as they were the concentrations which showed lesser physical changes and besides, they showed satisfactory antimicrobial effect on *L. monocytogenes*.

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