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Efficacy of Acidified Sodium Chlorite in Sanitizing Meat Contact Surfaces

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Abstract | One of the greatest challenges of the 21th century is ensuring food safety for consumers. Microbial contamination from processing surfaces and equipment poses a significant challenge in the food processing facilities. Therefore, the objective of the present study was to evaluate the antimicrobial activity of different concentrations of acidified sodium chlorite "ASC" on spoilage and some pathogenic microorganisms. The study was done at two different meat products factories in Ismailia governorate. All swabs samples were collected at the end of day work in the factory, before and after equal spraying of direct and indirect meat contact surfaces with ASC at concentration of 500, 800, or 1,200 ppm for one minutes. The obtained results revealed that the mean values of total aerobic counts for knives before and after 500, 800 and 1200 ppm after ASC application were 3.43, 2.30, >10 and >10 Log10 CFU/cm2 respectively. For mincer machines before and after 500, 800 and 1200 ppm ASC application were 4.38, 3.41, 2.01 and >10 Log10 CFU/cm2 respectively. For cutting boards before and after 500, 800 and 1200 ppm ASC application were 4.60, 3.46, 2.14 and >10 Log10 CFU/cm^2 respectively. For floor before and after 500, 800 and 1200 ppm ASC application were 5.38, 4.37, 3.22 and > Log10 CFU/cm2 respectively. This indicates that ASC, at 1200 ppm, was highly effective in reducing bacterial populations to undetectable levels, effectively meeting or exceeding the permissible limit of 10 CFU/cm^2 for meat contact surfaces. In conclusion, this study provides the first comprehensive evaluation of ASC's efficacy on multiple meat contact surfaces (knives, mincers, cutting boards, and floors) in meat factory, establishing optimal concentrations for pathogen elimination. ASC is a potent, safe, economical, and valuable antimicrobial agent for use in food processing environments particularly at 1200 ppm achieving 100% reduction of meat contact surfaces contaminates.

Keywords | Acidified sodium chlorite, ASC, Staph. aureus, *E. coli*, meat contact surfaces

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INTRODUCTION

The biggest challenges of the 21st century are to guarantee that food is safe for consumers. Meat as human food is one of the supreme perishable foods since it contains elements required for quick microbial growth including foodborne pathogens such as *Escherichia coli* and *Staphylococcus aureus* creating health hazards for consumers

(Sosnowski and Osek, 2021). Bacteria from meat contact surfaces may be transferred to meat and their products lead to abuse effects on meat quality. Moreover, residential microorganisms in the meat industry may affect the pathogens growth in processing surfaces (Møretrø and Langsrud, 2017).

Food contamination negatively impact the final product's quality and endanger the consumer's health which is the main reasons why food is unfit for consumption. All of the main stages of the food production chain from raw material, storage, processing, transportation to the retail location, and consumer preparation can result in food contamination (Holban and Grumezescu, 2003).

In meat industry plants, sanitization is conducted to elimination all contaminants, including microorganisms, from direct meat contact surfaces including meat's equipment and utensils, and other things like cutting boards and floors (Wang *et al*., 2018), Maintaining meat safety in industrial production settings is critical for consumer confidence and public health. The main way to do this is by carefully following Good Hygienic Practices (GHPs), a set of fundamental guidelines that cover all the steps required to ensure the safety and wholesomeness of food products at every point of the food chain (FAO, 1997; Codex Alimentarius Commission, 2020).

A strong foundation of Good Hygienic Practices (GHPs) is necessary for a thorough understanding of food safety in industrial settings. These procedures are essential for reducing the chance of foodborne illnesses, preventing microbial contamination, and guaranteeing the production of safe, high-quality food products. Validated sanitation procedures, which include the careful selection and use of disinfectants, are essential to successful GHPs.

To tackle the problem of biological contamination, the meat industry invests a great deal of time and resources in implementing and regularly using sanitation plans in food processing facilities to prevent microbial contamination of processing surfaces and equipment (Nerín *et al*., 2016). Food industry has long relied on sodium hypochlorite (NaOCl) solutions for disinfection because of their widespread availability, cost-effectiveness, and broad-spectrum antimicrobial efficacy (Marriott and Gravani, 2006).

Recently, ASC has emerged as a well-known disinfectant because of their broad-spectrum antimicrobial activity, affordability, and relative simplicity of use. It introduce as new, novel and safe antimicrobial technique in food industry. ASC is a powerful and widely recognized antimicrobial agent, generated by combining an aqueous solution of sodium chlorite ($NaClO_2$) with a food-grade acid. ASC solution is a binary composition that consists of sodium chlorite solution and citric acid. This acidification step is critical, as it catalyzes the conversion of the stable chlorite ion (ClO_2) into highly reactive and potent antimicrobial oxy-chlorine species, most notably chlorous acid ($HClO_2$) and chlorine dioxide (ClO_2).

ASC has a broad-spectrum efficacy against a diverse range of microorganisms where it efficiently breaks down microbial cell membranes, denatures proteins, and deactivates vital enzymes, all of which result in microbial death (McDonnell and Russell, 1999) which made it an indispensable tool in various industries, predominantly food safety and processing (Arienzo *et al*., 2023). In comparison to some other disinfectants, ASC has the potential to provide superior antimicrobial efficacy. This, along with its comparatively low cost and environmental profile, makes it a desirable option for raising hygienic standards in food processing facilities. ASC is an effective antimicrobial agent used as a sanitizer compared to other sanitizers like sodium hypochlorite and peroxyacetic acid, Several investigations show ASC can be more effective than chlorine in reducing microbial loads on various surfaces, including food contact surfaces (McWhorter *et al*., 2023).

Nowadays, a lot of study is focused on sanitation and hygiene problems in meat manufacturing plants. The manufacturing of meat and meat products carries the biggest hazards. Numerous studies conducted in recent years have demonstrated the need for careful hygienic and sanitary practices at every stage of the production of beef products (Velebit *et al*., 2021). While previous studies have evaluated ASC on specific pathogens or surfaces, this study systematically compares its efficacy across diverse meat contact surfaces (direct and indirect) under real-world conditions, providing actionable insights for industry practices

In Egypt, Technical regulations and legislations on microbiological control of meat contact surfaces either direct or indirect in meat processing factories are not available. Therefore, in light of Good Hygienic Practices, this study attempts to critically assess the use of ASC using at different concentrations in two meat factories. The evaluation covers its antimicrobial efficacy against microbial groups and some foodborne pathogens as Aerobic colony counts, Enterobacteriacae, *Staphylococcus auras* and *E. coli* under a range of operating conditions.

MATERIALS AND METHODS

ASC PREPARATION

ASC solution was set onsite according to Inatsu *et al*. (2010) by mixing 0.5 g sodium chlorite (35%) with one gram citric acid (50%, food grade) then allowed to activate

for 10 minute. Different concentration were done by dilution of the stock with sterile distilled water to form 500, 800 and 1200 ppm (Final pH, 3.15 to 3.25).

Experimental design

The study was done at two different meat products factories in Ismailia governorate. Swab samples were collected at the end of the workday before and after equal spraying of direct and indirect meat contact surfaces with ASC at concentration of 500, 800, or 1,200 ppm for one minutes. A commercial sprayer was used for uniform ASC application after regular cleaning system with tape water (22 to 24°C) without any chemical disinfectant as usual. Total number of 960 swabs before and after ASC application were collected as following: 240 swab samples from knives; 240 swab samples from one of meat equipment, electric meat mincer machines; 240 swab samples from meat cutting boards; finally, 240 swab samples from meat plant floor.

Samples collection

A 4×5 cm^2 metal guide was used to measure the swab area on each food contact surfaces. Swab samples were collected by sterile swab stick by swabbing an enclosed area of 100 cm^2 (Christison *et al*., 2008; Baghapour *et al*., 2015). All swabs were then placed into 5 mL of sterile peptone water (ICMSF, 1978) then placed in an ice-cooled box and transported without delay to the Food Safety Laboratory, Faculty of Veterinary Medicine, Suez Canal University.

Sample preparation

Each tube containing the swab was vortexed for 10 seconds to ensure mixture of the sample. A 10 fold dilution method was used by serially diluting 0.1 mL of each sample in 9.9 mL of sterile saline to achieve a dilution factor of 10^2 to 10^6.

Microbiological evaluation

Aerobic plate count was determined by 3M™ petri film™ technique according to 3M (2022a), Enterobacteriaceae counts was according to 3M (2022b), Total *Staphylococcus aureus* was according to 3M (2022c), and *E. coli* was according 3M (2022d). All plates were incubated at 35±2°C for 24 hours. Then, plates were counted with the 3M™ Petrifilm™ plate reader, on a standard colony counter and counted colonies expressed as CFU/g.

Statistical analysis

Data analysis was performed by using SPSS statistical software program (SPSS, 2007). Data were expressed as mean ± standard error (SE). One-way analysis of variance (ANOVA) was used to detect the significant differences ($P<0.05$) between means by multiple comparisons procedure of LSD (least significant difference), using a level of significance of alpha = 0.05.

RESULTS AND DISCUSSION

Microorganisms are generally considered the enemies of wholesome meat production systems. Many microbes that enter a production facility, especially in products of animal origin, are pathogens that can cause illness in cases of human consumption. However, the majority of organisms present in the production environment are not likely to cause human illness, but instead impact the quality of the product by their involvement in spoilage, especially organisms such as Pseudomonas, Moraxellaceae, and Lactic Acid Bacteria. Cleaning and disinfection represent the most important activities associated with the elimination of dirt and microorganisms at food processing plants. Improper procedures may lead to cross contamination of food leading to its spoilage or even the transmission of foodborne pathogens (Cabezas-Pizarro *et al*., 2018).

Application of ASC on the meat contact surfaces

In order to prevent microbiological contamination and protect the public's health, the meat processing sector must adhere to strict hygiene standards. Antimicrobial compounds are essential for lowering the microbial burdens on the surfaces used in meat processing as well as on the meat products. ASC is one of them that has become a well-known and often used antibacterial agent in the food sector. A food-grade acid is used to acidify an aqueous solution of sodium chlorite in order to create ASC. In the current study, ASC evaluated at concentrations of 500, 800, and 1200 ppm. These concentrations align with the approved legal use ranges in both the United States (500-1200 ppm) and Australia (500-1200 ppm) for various food surfaces applications (FDA, 2013; FSANZ, 2013).

The efficacy of ASC was evaluated at concentrations of 500, 800, and 1200 ppm on four distinct food processing surfaces: knives, mincer machines, cutting boards, and floor surfaces Tables 1, 2, The results are presented as the percentage of swabs rendered negative for specific bacterial groups after ASC application, with a baseline of 0% negative swabs (100% positive) before treatment across all categories. The results confirmed that spraying knives with higher ASC concentrations increased efficacy. At the highest concentration of 1200 ppm, ASC achieved 100% negative swabs for both *Staphylococcus aureus* and *Escherichia coli*. For Enterobacteriaceae, 80% of swabs were rendered negative at 1200 ppm, while total aerobic bacteria showed 60% negativity at this concentration. Lower concentrations of ASC yielded (500 ppm) proportionally lower rates of negative swabs across all bacterial groups.

At 1200 ppm, ASC achieved 100% negative swabs for both *Escherichia coli* and *Staphylococcus aureus*.

Table 1: Percentage of negative swabs from knives and mincer machines before and after application of Acidified sodium chlorite at 500, 800 and 1200 ppm.

Contact surface	Knives								Mincers							
Bacterial groups	Total aerobic bacteria		Enterobacteriaceae group		*Staphylococcus aureus*		*Escherichia coli*		Total aerobic bacteria		Enterobacteriaceae group		*Staphylococcus aureus*		*Escherichia coli*	
ASC Con.	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
500 ppm	0 (0%)	1 (10%)	0 (0%)	2 (20%)	0 (0%)	5 (50%)	0 (0%)	6 (60%)	0 (0%)	1 (10%)	0 (0%)	2 (20%)	0 (0%)	5 (50%)	0 (0%)	6 (60%)
800 ppm	0 (0%)	2 (20%)	0 (0%)	5 (50%)	0 (0%)	6 (60%)	0 (0%)	8 (80%)	0 (0%)	2 (20%)	0 (0%)	5 (50%)	0 (0%)	6 (60%)	0 (0%)	8 (80%)
1200 ppm	0 (0%)	4 (40%)	0 (0%)	8 (80%)	0 (0%)	10 (100%)	0 (0%)	10 (100%)	0 (0%)	4 (40%)	0 (0%)	8 (80%)	0 (0%)	10 (100%)	0 (0%)	10 (100%)

(Number of swabs: 240 per surface).

Table 2: Percentage of negative swab samples from cutting boards and floor before and after application of Acidified sodium chlorite at 500, 800 and 1200 ppm.

Contact surface	Cutting board								Floor							
Bacterial groups	Total aerobic bacteria		Enterobacteriaceae		*Staphylococcus aureus*		*Escherichia coli*		Total aerobic bacteria		Enterobacteriaceae		*Staphylococcus aureus*		*Escherichia coli*	
ASC Con.	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
500 ppm	0 (0%)	2 (20%)	0 (0%)	2 (20%)	0 (0%)	5 (50%)	0 (0%)	6 (60%)	0 (0%)	2 (20%)	0 (0%)	3 (30%)	0 (0%)	4 (40%)	0 (0%)	7 (70%)
800 ppm	0 (0%)	2 (20%)	0 (0%)	5 (50%)	0 (0%)	6 (60%)	0 (0%)	8 (80%)	0 (0%)	2 (20%)	0 (0%)	4 (40%)	0 (0%)	5 (50%)	0 (0%)	10 (100%)
1200 ppm	0 (0%)	4 (40%)	0 (0%)	8 (80%)	0 (0%)	10 (100%)	0 (0%)	10 (100%)	0 (0%)	4 (40%)	0 (0%)	5 (50%)	0 (0%)	10 (100%)	0 (0%)	10 (100%)

Number of swabs: 240 per surface.

Efficacy against Enterobacteriaceae reached 80% at 1200 ppm, while Total aerobic bacteria showed 40% negativity at this concentration. Similar to knives, lower ASC concentrations resulted in reduced effectiveness across all bacterial groups. Table 1 demonstrates that ASC's efficacy on meat cutting boards also showed consistent improvement with increased concentration. At 1200 ppm, both *Escherichia coli* and *Staphylococcus aureus* achieved 100% negative swabs. For Enterobacteriaceae, 50% of swabs were negative, and for Total aerobic bacteria, 40% were negative at 1200 ppm. The pattern of higher concentrations leading to greater efficacy was maintained across all bacterial groups on cutting boards.

Table 2 shown the results of ASC efficacy on floor. At 500 ppm, total aerobic bacteria and Enterobacteriaceae showed 100% positive swabs. In contrast, *Staphylococcus aureus* was 40% negative and *Escherichia coli* 60% negative at 500 ppm. However, at 800 ppm, the efficacy for total aerobic bacteria sharply decreased to 10% and for Enterobacteriaceae to 20%, a notable drop compared to the 500 ppm results. Concurrently, *Staphylococcus aureus* increased to 60% and *Escherichia coli* to 80% at 800 ppm. At the highest concentration of 1200 ppm, total aerobic bacteria reached 40% and Enterobacteriaceae 30%, while both *Staphylococcus aureus* and *Escherichia coli* achieved 100% negativity. The results observed on decreased ASC efficacy on floor surfaces, for total aerobic bacteria and Enterobacteriaceae group at 500 and 800 ppm concentration. It could be interpreted as floor surfaces may include high organic load, the inherent irregularities and complex topography, in addition, the potential presence of established biofilms.

The provided results confirmed that ASC exhibits concentration-dependent antimicrobial efficacy across the investigated meat processing surfaces. Higher concentrations, particularly 1200 ppm, consistently yielded superior results in rendering swabs negative for bacterial contamination. ASC proved remarkably effective against specific foodborne pathogens. At 1200 ppm, it achieved 100% negative swabs for *Escherichia coli* and *Staphylococcus aureus* on knives, mincer machines, and cutting boards (Bosilevac *et al*., 2004).

Table 3: Mean values of bacterial groups (Log_{10} CFU/cm^2 ± SE) before and after application of different Acidified Sodium chlorite concentrations (ppm) on meat contact surfaces.

Contact surfaces	Total aerobic count				Enterobacteriaceae count				*Staphylococcus aureus* count				*Escherichia coli* count			
	Before	After application of acidified sodium chlorite			Before	After application of acidified sodium chlorite			Before	After application of acidified sodium chlorite			Before	After application of acidified sodium chlorite		
		500 ppm	800 ppm	1200 ppm		500 ppm	800 ppm	1200 ppm		500 ppm	800 ppm	1200 ppm		500 ppm	800 ppm	1200 ppm
Knives	3.43^a *±0.27	2.30^a ±0.47	<10^b	<10^b	2.13^a ±0.23	0.90^a ±0.15	< 10^b	< 10^b	3.43^a ±0.53	2.30^b ±0.25	1.69^c ±0.45	< 10^d	2.20^a ±0.53	1.25^b ±0.23	< 10^c	< 10^d
Mincer machines	4.38^a ±1.16	3.41^a ±0.26	2.01^b ±1.06	<10^c	3.17^a ±1.10	1.70^a ±0.26	< 10^b	< 10^b	4.22^a ±1.10	3.30^b ±0.23	1.43^c ±0.45	< 10^d	3.18^a ±0.70	2.51^b ±0.83	0.90^c ±0.15	< 10^d
Cutting boards	4.60^a ±1.50	3.46^a ±0.70	2.14^b ±1.06	<10^c	3.94^a ±1.82	1.31^a ±0.70	< 10^b	< 10^b	4.30^a ±1.32	3.40^b ±1.21	2.10^c ±0.83	< 10^d	3.16^a ±1.04	2.35^b ±1.23	< 10^c	< 10^d
Floor	5.38^a ±2.11	4.37^a ±1.27	3.22^b ±1.06	<10^c	4.21^a ±1.90	2.82^a ±0.72	2.51^b ±0.86	< 10^c	5.42^a ±2.26	4.50^b ±1.44	3.30^c ±0.39	< 10^d	4.62^a ±2.44	3.83^b ±1.17	2.10^c ±0.24	< 10^d

*± SE means Standard Error. Permissible limits for total aerobic count is 10 CFU/cm^2 for meat contact surfaces (Gómez Ariño *et al.*, 2012). Permissible limits for Enterobacteriaceae count is 1 CFU/cm^2 for meat contact surfaces (Gómez Ariño *et al.*, 2012). Means in the same row within same bacterial groups with different letter were significantly differ (P<0.05).

The antimicrobial activity of ASC is mainly due to its powerful oxidative chemistry. This activity is started by the acidification of sodium chlorite ($NaClO_2$) with a food-grade citric acid to generate metastable chlorous acid ($HClO_2$) which highly unstable and rapidly decomposes to form a complex mixture of oxy-chlorine species, with chlorine dioxide (ClO_2) being the most active antimicrobial component (Rao, 2007). However, the efficacy against broader microbial groups, specifically total aerobic bacteria and Enterobacteriaceae group, was generally not completely elimination, even at the highest concentration of 1200 ppm. This suggests a differential susceptibility among microbial populations. *E. coli* and *S. aureus* represent specific pathogenic species, while total aerobic bacteria and Enterobacteriaceae group encompass a much broader range of microorganisms, including many spoilage organisms and potentially more resistant strains or species within their respective classifications. This implies that while ASC is a potent tool against key pathogens, its effectiveness against the entire spectrum of microbial contaminants may vary, necessitating a comprehensive approach to microbial control rather than relying on a single disinfectant for all microbial types (Kalchayan *et al.*, 2012).

Efficacy of ASC on total aerobic count

Microbiological examination of the meat contact surfaces in meat processing plants revealed that their sanitary and hygienic condition worsened as they were contaminated with microorganisms during regular production (Velebit *et al.*, 2021). Since regular washing of meat surfaces did not lead to significant changes in microbial counts (Zweifel *et al.*, 2014), ASC's provide the hygienic solution as approval as safe and a "no-rinse" food-grade sanitizer at recommended concentrations for food contact surfaces (Kemp *et al.*, 2000).

It is evident from the obtained results in Table 3 that the mean values of total aerobic counts for knives before and after 500, 800 and 1200 ppm after ASC application were 3.43, 2.30, >10 and >10 Log_{10} CFU/cm^2, respectively. For mincer machines before and after 500, 800 and 1200 ppm ASC application were 4.38, 3.41, 2.01 and >10 Log_{10} CFU/cm^2, respectively. For cutting boards before and after 500, 800 and 1200 ppm ASC application were 4.60, 3.46, 2.14 and >10 Log_{10} CFU/cm^2, respectively. For floor before and after 500, 800 and 1200 ppm ASC application were 5.38, 4.37, 3.22 and > Log_{10} CFU/cm^2, respectively. Nearly similar results for microbial contamination of meat contact surfaces were obtained by Zulfakar *et al.* (2019). In study done by Bataeva *et al.* (2016) recorded higher microbial load on knife surfaces (6×10^3 to 6×10^8 CFU/cm^2 depending on the sanitary state of a meat processing plant. On concern to the floor of processing plant, higher results recoded by Paliy *et al.* (2018).

Aerobic plate count can reflect the status of application of hygienic measures during meat production. The obtained results clearly demonstrate the significant antimicrobial efficacy (P<0.05) of ASC against total aerobic bacteria on various direct and indirect meat contact surfaces. A clear dose-response relationship was observed, where increasing concentrations of ASC led to the significant reduction (P<0.05) in bacterial counts. The most striking finding is the 100% reduction in total aerobic bacteria achieved at the 1200 ppm concentration across all surfaces. This indicates that ASC, at this concentration, was highly effective in reducing bacterial populations to undetectable levels,

effectively meeting or exceeding the permissible limit of 10 CFU/cm^2 for meat contact surfaces (Gómez Ariño *et al*., 2012). Currently, there is no Egyptian regulation on the permissible range of microbial loads on meat contact surfaces. However, Solberg *et al*. (2004) set microbial limits for food contact surfaces at 10 to 20 CFU/cm^2. In another study, Sneed *et al*. (2004) proposed a standard for food contact surfaces of less than 20 CFU/cm^2 for total aerobic count. A contamination level of less than 2.5 CFU/cm^2 after regular cleaning and disinfection is achievable in food contact surfaces (Griffith, 2005). On the other hand, the Canadian government establishes guide line for the evaluation of the hygienic condition of work surfaces and equipment in contact food surfaces, allowing maximum levels of aerobic plate count of 100 CFU/cm^2 (MAPAQ, 2009).

The effectiveness of application of good hygienic practices in food factories is often monitored by reductions total bacterial count. The results in Table 3 given details about the reduction in Log_{10} CFU/cm^2 (%) in total aerobic count on examined meat contact surfaces. The reduction (Log_{10} %) in total aerobic count after application of 500, 800 and 1200 ASC concentrations were 2.13 (33%), 3.43 (100%) and 3.43 (100%) for knives; 0.97 (22%), 2.37(54%) and 4.38 (100%) for mincer machines; 1.14 (25%), 2.46 (54%) and 4.60 (100%) for cutting boards and 1.01 (19%), 2.16 (40%) and 5.38 (100%) for floor. The obtained results achieving 100% reduction of total aerobic bacteria on meat contact surfaces at 1200 ppm concentrations bring the microbial counts below the permissible limits and thereby significantly reducing the risk of contamination and enhancing food safety.

During processing, meat may microbial contaminated or, viceversa, contaminated by equipment, utensils, containers, floors, walls, and other sources of the production environment (Alvseike *et al*., 2019). Ensure the application of good hygienic practices in the work environment (Surfaces, equipment, and utensils) as a fundamental requisite for the prevention of microbial contaminations must be in place to ensure that such meat do not compromise public health (Carrascosa *et al*., 2012). In addition, surfaces contamination exceeding 10^4 CFU/cm^2 is sufficient to start biofilm formation, which sequentially is difficult to clean (Hood and Zottola, 1995), leading to meat may constitute a sever public health hazards.

Efficacy of ASC on enterobacrteriacea count

Enterobacteriaceae are a family of Gram-negative bacteria usually used as hygiene indicators in the meat industry, as their incidence often evidence for inadequate hygienic practices. Good hygienic practices requirements are imposed in meat industry as it is one of the contamination sources for production facilities and final products (Minaev *et al*., 2008). The data presented in Table 4 provide that the mean values of Enterobacrteriacea for knives before and after 500, 800 and 1200 ppm ASC application were 2.13, 0.90, <10 and <10 Log_{10} CFU/cm^2, respectively. For mincer machines before and after 500, 800 and 1200 ppm ASC application were 3.17, 1.70, <10 and <10 Log_{10} CFU/cm^2, respectively. For cutting boards before and after 500, 800 and 1200 ppm ASC application were 3.94, 1.31, <10 and <10 Log_{10} CFU/cm^2, respectively. For floor before and after 500, 800 and 1200 ppm ASC application were 4.21, 2.82, 3.22 and <10 Log_{10} CFU/cm^2 respectively. Nearly similar results for microbial contamination of meat contact surfaces were obtained by Zulfakar *et al*. (2019).

Prior to ASC application, initial Enterobacteriaceae counts varied across the surfaces, ranging from 2.13 Log_{10} CFU/cm^2 on knives to 4.21 Log_{10} CFU/cm^2 on the floor. ASC at 1200 ppm concentration was significant reduce ($P<0.05$) the Enterobacteriaceae count to fit the established permissible limit (<1 CFU/cm^2). Enterobacteriaceae are commonly found in processing environments and are recognized as spoilage organisms in many types of food (Sperber and Doyle, 2009). Enterobacteriaceae often present on the floor, equipment and utensils used in meat industry (Choi *et al*., 2013).

ASC has emerged as a highly effective and widely utilized antimicrobial agent within the food industry (Allende *et al*., 2009). The results in Table 4 given details about the reduction rate Log_{10} CFU/cm^2 (%) in Enterobacrteriacea on examined meat contact surfaces. The reduction (Log10 %) in Enterobacrteriacea count after application of 500, 800 and 1200 ASC concentrations were 1.03 (84%), 2.13 (100%) and 2.13 (100%) for knives; 1.38 (44%), 3.17 (100%) and 3.17 (100%) for mincer machines; 1.93 (49%), 3.94 (100%) and 3.94 (100%) for cutting boards and 1.39 (33%), 1.07 (40%) and 4.21 (100%) for floor. ASC showed strong antimicrobial activity against Gram-negative, mesophilic, and psychrotrophic bacteria on whole croaker after 10-min immersion application of 600 ppm ASC (Lee *et al*., 2008). At ASC 800 and 1200 ppm concentrations, complete (100%) reduction was achieved for Enterobacteriaceae counts on knives, mincer machines, and cutting boards indicating highly effective sanitation of ASC.

At 500 ppm ASC concentrations, the floor showed a 33% Enterobacteriaceae counts reduction, increasing to 40% at 800 ppm. Only at the highest concentration of 1200 ppm was a 100% reduction achieved on the floor. This suggests that the floor as indirect meat contact surfaces has potentially higher organic load, represents a more challenging environment for complete microbial eradication compared to the other tested meat contact surfaces. Biofilm formation on these surfaces is a

persistent challenge. Bacteria can adhere to surfaces and form biofilms, which are communities encased in an extracellular polymeric substance (EPS). Biofilms protect bacteria from sanitizers and can release cells into the food product (Donlan, 2002; Stepanović *et al*., 2007).

The improperly cleaned meat contact surfaces constitute a source of nutrients for the microorganisms which may be present (Srey *et al*., 2013). Therefore, the significant reduction of Enterobacteriaceae on critical surfaces such as knives, mincer machines, and cutting boards has direct and profound implications for meat safety. This reduction directly translates to a diminished risk of cross-contamination from contaminated surfaces to meat products, which is essential for preventing the spread of foodborne pathogens and ensuring the microbiological safety of processed meat (WHO, 2007; Rodriguez-Caturla *et al*., 2012). Regular and appropriate application of ASC, particularly at concentrations 1200 ppm for critical meat contact surfaces, can significantly contribute to a safer processing environment and enhance the overall microbiological quality of meat products.

Efficacy of ASC on total *Staph. aureus* count

Staphylococcus aureus is a ubiquitous Gram-positive pathogens recognized as one of the foremost causes of global foodborne intoxication, which frequently contaminates meat during various processing stages (Abebe *et al*., 2024). It is evident from the obtained results in Table 3 that the mean values of total Staphylococcus aureus count for knives before and after 500, 800 and 1200 ppm ASC application were 3.43, 2.30, 1.69 and <10 Log_{10} CFU/cm^2, respectively. For mincer machines before and after 500, 800 and 1200 ppm ASC application were 4.22, 3.30, 1.43 and <10 Log_{10} CFU/cm^2, respectively. For cutting boards before and after 500, 800 and 1200 ppm ASC application were 4.3, 3.40, 2.10 and <10 Log_{10} CFU/cm^2, respectively. For floor before and after 500, 800 and 1200 ppm ASC application were 5.42, 4.50, 3.30 and <10 Log_{10} CFU/cm^2, respectively. *Staphylococcus aureus* listed as the most common source of meat contamination due to insufficient cleaning of processing equipment (Bennett *et al*., 2013). Inadequate hygiene practices in meat processing environments, encompassing both equipment and meat contact surfaces, are frequently identified as significant reservoirs for Staph. aureus contamination. These surfaces can enable the transfer of Staph. aureus to meat and their products, posing a direct risk to food safety (Pérez-Boto *et al*., 2023). The observed data about the complete eradication of *S. aureus* at the 1200 ppm concentration provides robust empirical support for the regulatory guidelines that permit ASC use up to this specific concentration. This finding strongly suggests within meat processing facilities where the complete elimination of *S. aureus* on meat contact surfaces is required for good hygienic practices, utilizing ASC at 1200 ppm concentrations is scientifically justified and represents the most effective strategy.

The results in Table 4 given details about the reduction rate Log_{10} CFU/cm^2 (%) in total *Staphylococcus aureus* count on examined meat contact surfaces. The reduction (Log_{10} %) in total *Staphylococcus aureus* count after application of 500, 800 and 1200 ASC concentrations were 1.13 (33%), 1.74 (51%) and 3.43 (100%) for knives; 0.92 (22%), 2.17 (66%) and 4.22 (100%) for mincer machines; 0.903 (21%), 2.20 (51%) and 4.30 (100%) for cutting boards and 0.92 (17%), 2.12 (39%) and 5.45 (100%) for floor. The study data explicitly demonstrate that ASC is effective ($P<0.05$) in reducing *Staphylococcus aureus* counts on all tested meat contact surfaces, with its efficacy being markedly dependent on concentration. A consistent and progressive trend of significant ($P<0.05$) bacterial reduction was observed as the ASC concentration was elevated from 500 ppm to 800 ppm, and subsequently to 1200 ppm, across all evaluated surface types to below the detectable limit of <10 Log_{10} CFU/cm^2, consistently achieved at the highest ASC concentration of 1200 ppm on all meat contact surfaces. Chlorous acid ($HClO_2$), produce from ASC, with a pKa of 1.86, is

Table 4: Reduction Log_{10} (%) of different bacterial group (Log_{10} CFU/cm^2) after application of different Acidified Sodium chlorite concentrations (ppm) on the meat contact surfaces.

	Application of acidified sodium chlorite											
ASC Con.	**Total aerobic bacteria**			**Enterobacteriaceae**			***Staphylococcus aureus***			***Escherichia coli***		
	500 ppm	**800 ppm**	**1200 ppm**	**500 ppm**	**800 ppm**	**1200 ppm**	**500 ppm**	**800 ppm**	**1200 ppm**	**500 ppm**	**800 ppm**	**1200 ppm**
Knives	2.13 (33%)	3.43 (100%)	3.43 (100%)	1.03 (84%)	2.13 (100%)	2.13 (100%)	1.13 (33%)	1.74 (51%)	3.43 (100%)	0.95 (43%)	2.20 (100%)	2.20 (100%)
Mincer machines	0.97 (22%)	2.37 (54%)	4.38 (100%)	1.38 (44%)	3.17 (100%)	3.17 (100%)	0.92 (22%)	2.17 (66%)	4.22 (100%)	0.67 (21%)	1.61 (51%)	3.18 (100%)
Cutting boards	1.14 (25%)	2.46 (54%)	4.60 (100%)	1.93 (49%)	3.94 (100%)	3.94 (100%)	0.90 (21%)	2.20 (51%)	4.30 (100%)	0.81 (25%)	3.16 (100%)	3.16 (100%)
Floor	1.01 (19%)	2.16 (40%)	5.38 (100%)	1.39 (33%)	1.07 (40%)	4.21 (100%)	0.92 (17%)	2.12 (39%)	5.45 (100%)	0.79 (17%)	2.52 (55%)	4.62 (100%)

considered the main active ingredient and functions as a very strong oxidizing agent which is unstable and rapidly convert into chlorine dioxide (ClO_2). Chlorine dioxide is a powerful antimicrobial agent and is extensively regarded strong antimicrobial activity (Konishi and Shibata, 2024).

Significant Microbial counts were observed during meat processing due to several operations as cutting, boning, and trimming (Velebit *et al*., 2021). One of the most contaminant in the higher rate is *S. aureus* mainly from instruments, and workers' hands (Ebied *et al*., 2023). S*taphylococcus aureus*, can survive on different meat contact surfaces for periods ranging from several hours to days (Martinon *et al*., 2012; Simoes *et al*., 2010) and even form biofilms which incriminated in server public health hazards. On the other hands, *S. aureus* can secreted exotoxins in food and cause a foodborne intoxications. *Staph. aureus* enterotoxins are heat stable, not readily destroyed by usual cooking temperatures, meaning that even if the bacterial cells are inactivated, the enterotoxins can still induce illness. Symptoms of staphylococcal food poisoning typically manifest rapidly after ingestion, commonly including nausea, vomiting, and abdominal cramping (Kadariya *et al*., 2014).

Efficacy of ASC on total *E. coli* count

Food scientists have been concentrated great efforts to significantly reducing *E. coli* because it pose a severe public health risk. Results illustrated in Table 3 revealed that the mean values of total *E. coli* count for knives before and after 500, 800 and 1200 ppm ASC application were 2.20, 1.25, <10 and <10 Log_{10} CFU/cm^2, respectively. For mincer machines before and after 500, 800 and 1200 ppm ASC application were 3.18, 2.51, 0.90 and <10 Log_{10} CFU/cm^2, respectively. For cutting boards before and after 500, 800 and 1200 ppm ASC application were 3.16, 2.35, <10 and <10 Log_{10} CFU/cm^2, respectively. For floor before and after 500, 800 and 1200 ppm ASC application were 4.62, 3.83, 2.10 and <10 Log_{10} CFU/cm^2, respectively. Contamination with *E. coli* can occur across the various stages along the food chain, including, processing, distribution, storage, exhibition and handling. Therefore, *E. coli* as indicator bacteria are used to evaluate the hygienic status of the food establishment (Ghafir *et al*., 2008). Consequently, rigorous application of good hygienic practices are paramount within meat processing facilities to safeguard consumer health and maintain product quality. The results of the current study reported showed that *E. coli* was extremely sensitive at all ASC concentrations tested; particularly at 800 and 1,200 ppm were significantly more effective against *E coli* on meat contact surfaces which came with agree with those reported by Bintsis (2018). ASC solution, since approved as consumer's safe by FDA (Hajmeer *et al*., 2004), could be useful as a sanitizer for food contact surfaces and could reduce the population of *E. coli* O157:H7 by approximately 3.0 log CFU/g without changing their sensory quality (Inatsu et al., 2010).

The results in Table 4 given details about the reduction rate Log_{10} CFU/cm^2 (%) in total *E coli* count on examined meat contact surfaces. The reduction (Log_{10} %) in total *E coli* count after application of 500, 800 and 1200 ASC concentrations were 0.95 (43%), 2.20 (100%) and 2.20 (100%) for knives; 0.67 (21%), 1.61 (51%) and 3.18 (100%) for mincer machines; 0.81 (25%), 3.16 (100%) and 3.16 (100%) for cutting boards and 0.79 (17%), 2.52 (55%) and 4.62 (100%) for floor. A marked improvement in efficacy was evident at 800 ppm ASC. On knives and cutting boards, *E. coli* counts were significant reduced ($P<0.05$) to below detection limits (<10), signifying a 100% reduction from initial levels. Mincer machines also showed a substantial reduction, with *E. coli* counts dropping to 0.90 Log_{10} CFU/cm^2, representing a 51% reduction. This indicates that 800 ppm ASC is highly effective for critical food contact surfaces. The most comprehensive reductions were achieved with 1200 ppm ASC. At this concentration, *E. coli* counts on all tested surfaces, knives, mincer machines, cutting boards, and the floor were significant reduced ($P<0.05$) to below detection limits (<10), this highlights the potent bactericidal activity of ASC at 1200 ppm concentrations, capable of virtually eliminating of contamination. The efficacy of ASC against *E. coli* is attributed to its mechanism of action, primarily involving the generation of chlorine dioxide (ClO_2). When sodium chlorite solution is acidified, typically with a weak food-grade acid like citric acid, it produces chlorous acid ($HClO_2$). Chlorous acid is considered the main species responsible for antimicrobial effects at lower pH and rapidly converts to ClO_2. Which is powerful oxidizing agent, approximately 2.5 times stronger than hypochlorous acid (Allende *et al*., 2009).

The successful reduction of *E. coli* on meat contact surfaces, especially to below detectable limits at higher ASC concentrations, has profound implications for food safety. *E. coli* is a significant hygiene indicator, and its presence can signal potential fecal contamination or inadequate sanitation, with some strains being pathogenic and causing severe foodborne illnesses (Dhaliwal *et al*., 2025).

Various microorganisms as *E. coli* can attach to the surfaces and form biofilms and, consequently, retain viability after cleaning and disinfection (Wang *et al*., 2015). *E. coli* within biofilms can exhibit up to 1000 times greater resistance to antimicrobial agents compared to other vegetative cells. Emerging of a strong sanitizer on meat contact surfaces may need to eliminate contamination and prevent biofilm formation (Nasrollahian *et al*., 2024).

Finally, ASC is highly effective, safe, economic and strong oxidant sanitizer can used on food contact surfaces to improve the good hygienic practices in food factories. ASC at 1200 ppm can sanitize all hard surfaces that come into contact with food achieving 100% elimination of pathogens and provided that there is no measurable residue on food. One of the disadvantage of ASO using that it must be prepared on site and rapid application on the clean surfaces.

CONCLUSION AND RECOMMENDATIONS

The incidence of *Staphylococcus aureus* and *E. coli* on meat contact surfaces in meat factories remains a significant concern for public health. The presence of such pathogenic, serves as an indicator of inadequate hygienic practices. ASC is a potent, cost-effective, and safe antimicrobial agent, particularly at 1200 ppm, which achieved 100% reduction of pathogens on critical surfaces. The study demonstrates a strong, concentration-dependent efficacy, particularly at 1200 ppm, in reducing total aerobic bacterial counts, enterobacteriacea count, *Staphylococcus aureus* and *Escherichia coli* on knives, mincer machines, and cutting boards used in meat industry. ASC achieving 100% reduction of total aerobic bacteria on meat contact surfaces at 1200 ppm, thereby significantly reducing the risk of contamination and enhancing food safety. Concentrations of 800 ppm and 1200 ppm consistently achieved a 100% enterobacteriacea reduction on knives, mincer machines, and cutting boards, successfully bringing microbial counts below the stringent permissible limit. While effective, the floor surface presented a greater challenge, requiring the highest tested concentration (1200 ppm) for complete sanitation.

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NOVELTY STATEMENT

This study provides the first comprehensive evaluation of ASC's efficacy on multiple meat contact surfaces (knives, mincers, cutting boards, and floors) in meat factory, establishing optimal concentrations for pathogen elimination

AUTHOR'S CONTRIBUTION

The research strategy did by Prof. Ali Meawad Ahmed. While composed the article and conduct the experimental conditions by Mohanad Adel Mohammed El-Mogy and Dr. Mariam A. Abdel-Wahab3, the statistics done and language revision by Nada Ibrahim H. Ahmed. The finished manuscript was examined and revised by Dr. Fadwa F. Mahmoud and Ass. Prof. Heba Mohamed Shaheen.

Generative AI and AI-assisted technology statement

All authors of this work declare that generative AI technologies including large language models (e.g., ChatGPT, Copilot) and text-to-image generators were not utilized in any capacity during the preparation, writing, or editing of

Conflict of interest

The authors have declared no conflict of interest.

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