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Improving the Sanitary and Hygienic Conditions of Cold Smoking Fish Using Acidified Sodium Chlorite

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ABSTRACT

In large-scale food processing, food may be handled by many individuals, thereby there is an increase in the chances of food contamination due to abused handling. This study pointed to assess the food handlers' attitudes, knowledge and practices towards food safety in three fish cold-smoking plants in the Sharqiyah Government. The swabbing technique and bacterial analysis of processing plant surfaces monitored sanitary conditions. Then, acidified sodium chlorite-ASC- was used as an antimicrobial compound at concentrations of 500 and 1000 ppm to reduce bacterial contamination on the surfaces of cold-smoked fish. Along with the rapid screening of food-contact surfaces hygiene after the spray of ASC at concentrations of 500, 1000, and 1500 ppm using ATP-monitoring swabs. The yes-no questions surveys were conducted through face-to-face interviews with food handlers. The significantly low level of knowledge presented in this study suggests the inadequacy of the food safety training programs among food handlers. Good attitudes and practices of workers in smoked fish plants reflected the safety of the final product. The total aerobic plate count (APC) and halophilic bacterial counts of the smoked fish were 4.17± 0.42 and 3.01± 0.71 log CFU/g, respectively. The obtained microbial results were less than the levels allowed by the Egyptian standardization in concerns. In addition, the smoked fish was free from *Staphylococcus aureus* and *Listeria*. Surfaces might suggest the presence of insufficient cleaning during processing. The best reduction in APC at food contact surfaces was associated with the concentration of 1500 ppm ASC, compared to 500 and 1000 ppm concentrations. When ASC was applied directly to cold smoked fish, it reduced the APC from 4.95 CFU/g (control) to 4.34 and 4.04 logs CFU/g for 500 and 1000 ppm concentrations, respectively, without significant differences. Good knowledge of workers and adequate cleaning procedures with the application of ASC (500, 1000 and 1500 ppm) on food contact surfaces and up to 1000 ppm on smoked fish are needed to improve food safety in cold-smoking fish plants.

INTRODUCTION

Food is basic to maintain human survival, activities and development. With the progress of society, food safety has attracted more attention from consumers **(Zhao,**

2020). However, different factors such as highly toxic pesticides, harmful chemical substances, microbial contamination and the dishonest behavior of food producers directly affect food safety **(Huang & Liu, 2008)**. When food is cooked on a large scale, it may be handled by many persons and thus increasing the probability of contamination of the final food product. Unintended contamination of food leads to foodborne disease outbreaks, which can propose risks to the health of consumers and affect the economic costs for nations **(Adams & Motarjemi, 1999; Akabanda *et al*., 2017)**.

Foodborne-related illnesses have increased over the years, negatively affecting many developing nations' health and economic state **(WHO, 2007)**. About 1.8 million persons died from foodborne diseases in 2005, mainly due to ingesting contaminated food and drinking water **(WHO, 2007)**. The contamination arises from unhygienic practices, insufficient preservation methods, cross contamination from food contact surfaces or persons harboring the microorganisms **(Barrie, 1996; Jay *et al*., 1999)**. Unhygienic practices create prosperity conditions for foodborne pathogens **(Gent *et al*., 1999; Fielding *et al*., 2001; Akabanda *et al*., 2017)**. For example, numerous reported cases of foodborne viral diseases have been attributed to infected food handlers in catering services **(WHO, 1999; Bosch *et al*., 2018).**

The food handlers' attitudes, knowledge and practices have been reported in studies from different countries around the world **(Ansari-Lari *et al*., 2010; Seaman & Eves, 2010)**. This is because a combination of these three factors plays a leading role in food safety levels in the food processing industry **(Sharif & Al-Malki, 2010; Akabanda *et al*., 2017)**. Such studies are important as they evaluate training requirements, attitudinal changes and the efficiency of education and training. This, in turn, would impact consumer assurance of food safety.

Several outbreaks of foodborne diseases were reported associated with smoked fish **(Tham *et al*., 2000; Gillesberg Lassen *et al*., 2016)**. To prevent outbreaks of foodborne diseases in smoked fish plants, high standards of hygienic and safety practices by food handlers are essential parts of an overall food safety program implemented by these institutions. Although food handlers may have the required knowledge and skills needed in food safety practices, errors due to human handling are repeatedly cited in foodborne disease outbreaks **(Cieslak *et al*., 1996; Akabanda *et al*., 2017)**. The improper human handling of foods was stated in about 97% of food-poisoning cases **(Greig *et al*., 2007)**.

Bacterial contamination in ready-to-eat seafood, particularly cold-smoked products, is a safety concern. Cold-smoked fish are generally produced without intense thermal treatment and consumed without further heating **(Elliot & Kvenberg, 2000)**. *Listeria monocytogenes* can specifically multiply at refrigeration temperatures before products are consumed and reach significant levels that can cause listeriosis **(Guyer &**

Jemmi, 1991). The control of bacterial contamination would greatly reduce microbial hazards associated with the consumption of cold-smoked fish.

Acidified sodium chlorite -ASC- is an antimicrobic agent produced by mixing concentrated sodium chlorite with a generally-recognized-as-safe acid. ASC concentrations of 500 to 1,200 ppm were approved by the U.S. food and drug administration for use with poultry, red meat, comminuted meat products and processed fruits and vegetables to reduce bacterial contamination **(CFR, 1999)**. ASC solutions, 1200 ppm was effective in decontaminating beef, poultry carcasses, raw salmon and rainbow trout **(Kemp *et al*, 2000; Su & Morrissey, 2003; Beverly *et al*., 2006; Kamireddy *et al*., 2008)**. Yet, their application for decreasing bacterial contamination in processed cold-smoked fish and its effect on food-contact surfaces needs more investigation.

Maintaining the overall quality and safety of cold-smoked fish are priorities for intervention strategies that improve and extend the final product's shelf life and prevent foodborne outbreaks. This study, therefore, sought to evaluate the current situation in 3 cold-smoking plants in Sharqiyah Government, Egypt through the following steps: Initially, the food safety attitude, knowledge and behavior among workers in cold-smoking plants were evaluated using personal questionnaires. Bacterial analysis was conducted on the final cold-smoked fish product from previous processing plants. Bacterial analysis of all food contact surfaces in the processing plants followed the previous step. Then, the application of acidified sodium chlorite (ASC) antimicrobial was proposed to reduce bacterial contamination on the final product to improve the hygiene of food-contact surfaces. This purpose was assessed through a bacterial analysis of the final product after surface application of ASC in 500 and 1000 ppm concentrations. A rapid screen of food-contact surfaces hygiene was conducted after the spread of ASC in 500, 1000 and 1500 ppm conc. solutions using ATP-monitoring swabs.

MATERIALS AND METHODS

1. Questionnaire

1.1. Participants

Questionnaire surveys were performed on November 2020 in three cold-smoking processing plants in Sharqiyah Government, Egypt. Food processing plants surveyed in this work represented major cold-smoking processing plants in this area. A total of 25 workers directly participated in the processing of cold-smoked fish products were conveniently interviewed, and the response rate was 100%.

1.2. Data collection

The questionnaire was established based on the previous studies of **Akabanda *et al*. (2017)** and **Chen *et al*. (2018)**. Questionnaires were distributed among all workers at their rest and completed by personnel interviews. The questionnaire was done in 3 smoking plant workers and took about 10–15 min to complete. The questionnaire consisted of four sections including demographic information, knowledge, attitudes and practices about food safety. Demographic information included gender, age, education, duration of employment in the food processing industry by years, employment status, enrollment in food safety training courses and medical examination history.

Knowledge of food safety was assessed using 20 questions. The yes-no questions have two options (yes, he knew, and no, he did not know). A correctly answered question was given a score of 2 points, while a score of 1 was for incorrect ones, and the total scoring ranged from 20 to 40. Knowledge scores for individuals were counted and added up to obtain the total knowledge score. Participants who responded correctly were considered to have good food-safety knowledge.

Attitudes were evaluated with ten questions, and participants have three choices of agree, disagree, and unsure scored as 3, 2, and 1, respectively. The total score ranged from 10 to 30. Individuals' attitude scores were counted to acquire the total score. Participants who agreed were considered to have correct (positive) answers, while otherwise were considered to have a negative attitude toward food safety.

For twenty practice questions, participants have two options (yes, he did / no, he didn't). A correctly answered question was scored 2 points, while a score of 1 was for an incorrect one. The total score ranged from 20 to 40. Practice scores for individuals were counted and added up to get the total score. The actual responses were counted to estimate the minimum-maximum range. Differences between min and max actual results were used to estimate response categories as poor, average and good.

2. Bacterial analysis of surfaces swabs and smoked fish

2.1. Sampling

A total of 50 swabs were collected from 3 different smoking fish plants in Elsharkeia Government. In addition to the swabs, 10 random smoked fish (Herring) from packed final products were gathered. Swabs were kept in sterile test tubes, while fish were kept in sterile polyethylene bags. All samples were identified and then placed in an ice box and transferred to the Faculty of Fish Resources laboratories, Suez University under complete aseptic conditions. The collected samples were subjected to immediate analysis after arrival.

2.2. Bacterial analysis

Fish samples (5 g) represented by smoked fish (herring) were aseptically excised, weighed and transferred into stomacher bags containing 45ml of 0.1% (w/v) sterile buffered peptone water (Oxoid-CM509). The content was stomached using a Seward stomacher (400^R /UK) for 2 minutes. Such homogenate represents the dilution of 1:10 (10^{-1}) up to 4 dilutions. Further dilutions were spread plated at different media in duplicates. Total aerobic plate count enumerated on plate count agar media (Lab M, UK), and halophilic bacteria were counted using plate count agar with the addition of 5% sodium chloride. Plates were incubated at 35 ± 2 °C for 24h. Yeasts and molds were counted using potato dextrose media (Lab M, UK) and incubated at 25°C for 5d. Listeria selective media (Hi media, India) were used to count *Listeria* and were incubated at 35°C for 24h. *Staphylococcus aureus* bacteria were counted using Mannitol Salt Agar and Baird Parker Agar media **(APHA, 2001; FDA, 2001)**.

The cotton swabs were dipped into test tubes containing 1ml of peptone broth 0.1% and rubbed against the selected test area 10 cm x 10 cm. The count of bacteria was presented by log CFU/100 cm^2. When the subject is small, all its surface is swabbed **(Jones *et al*., 2020)**. Then, swabs were returned to the test tube containing the peptone broth. From this original sample, 1-10 serial dilutions were prepared up to 4 dilutions. As previously explained, total aerobic bacteria, *Staphylococcus aureus* count, and *Listeria* count were performed. Total coliform bacteria were counted using pour platting with tempered melted violet red bile agar medium (VRBA). *Salmonella* bacteria were enriched in selective tetrathionate broth and then plated on XLD agar, and selected colonies were confirmed with triple sugar iron, indole, methyl red, vogus-proskauer and citrate biochemical tests **(FDA, 2020)**.

3. Application of Acidified sodium chlorite Sanitizer on food-contact surfaces

3.1. Acidified sodium chlorite (ASC) preparation

Acidified sodium chlorite solution -ASC- was prepared by adding 1g/L of sodium chlorite with 0.5, 1 and 1.5 g/L citric acid, and then it was allowed to activate for 10 minutes. Sterile distilled water was added to form different dilutions of 500, 1000 and 1500 ppm concentrations. These treatments were freshly prepared just before the application **(Inatsu *et al*., 2010)**.

3.2. Surface application

The stainless-steel surface defined with 10x10 cm^2 areas was cleaned using a cleaning protocol. This involved removing large particles and soil from surfaces, premising with water to remove small particles **(Davidson *et al*., 1999)**. ASC sanitizer was sprayed on the selected areas as well as hot water and tape water (control) and was let to dry for 2 minutes.

3.3. ATP bioluminescence assay

The swab bud mobbed the entire 100cm^2 surface area and rotated constantly during swabbing. Each surface was swabbed in two opposite directions **(Moore *et al*., 2001)**. Swabs were used in the hygiene monitoring test kit (LuciPac®) that tested cleanliness levels, based on the evidence of ATP by manufacturer instructions. Detection of ATP indicates the presence of either microbial contaminants or food residues that might support microbial growth **(Volovyk *et al*., 2019; Miettinen *et al*., 2001)**.

3.4. Application on fish surface

Two concentrations of 500 and 1000 ppm ASC plus tape water (control) were applied separately on the surfaces of fish in triplicates. They were sprayed on the whole fish's surface and let to dry for 2 minutes. After the selected time, 5g of fish was cut and prepared for 1/10 serial dilutions for each treatment for aerobic bacterial count analysis. Dilutions were spread, plated on plate count agar media (Lab M, UK) and represented as CFU/g.

4. Statistical analysis

Descriptive analysis of questionnaire data including means, standard deviations (SD) and frequency percentages (%) was performed for all variables using IBM SPSS Statistics 25 software (IBM Corporation, NY, USA). Bacterial counts were compared using an independent sample t-test, and results were presented with means ± SD. Mean differences were compared by Duncan's multiple range test. A probability value (P) of less than 0.05 was used to indicate statistically significant differences.

RESULTS AND DISCUSSION

1. Questionnaire

The demographic information illustrated that all the participants were males, and most (64%) of them were from 21-30 years of age. Only 40% of them had high education with a college degree, and the majority (96%) did not take any food safety training courses. The average duration of employment was from 11-20 years and mostly (88%) part-time work. In addition, fifty-six percent of them do frequent medical examinations (Table 1).

Table 1. Demographic characteristics of the respondents

Variable		***Number****	***Percent %***
Gender	Male	25	100
	Female	0	0
Age	15-20	2	8
	21-30	16	64
	31-40	6	24
	41-50	1	4
Education	**Low** (not formal, primary or secondary)	15	60
	High (collage)	10	40
Length of employment/years	< 5	5	20
	5-10	5	20
	11-20	10	40
	21-30	5	5
Employment status	Full-time	3	12
	Part-time	22	88
Food safety training	Yes	1	4
	No	24	96
Do medical examination	Yes	14	56
	No	11	44

*Number of respondents

This study aimed to evaluate knowledge, attitudes and behavior towards food safety among cold-smoking fish processing plant workers. Results demonstrated that workers employed in the three cold-smoking fish in the Sharqiyah Government have a relatively poor level of knowledge, yet suitable attitudes and practices. About 45% (9/20 questions) of the cold-smoking fish plant workers answered correctly to more than 50% of the knowledge questions (Table 2). However, their knowledge of the specific diseases or pathogens that could transmit through cold-smoked fish products was quite low. Similarly, other studies showed that food processing workers did not have enough knowledge regarding foodborne diseases or pathogens **(Young *et al*., 2010; Akabanda *et al*., 2017; Chen *et al*., 2018)**. About 40% only knew that many types of bacteria can be present in food and cause health problems and chronic diseases (Q6: Table 2). Only 4% of the respondents were aware that the types of bacteria causing health problems are *Listeria*, *Salmonella* and *Staphylococcus* (Q7: Table 2). This means the majority of participants were unaware of this important knowledge. This might be due to the majority (96%) having low education (60%) and not receiving food safety training, (Table 1).

Positive attitudes were reported by 56% of the workers, respectively (Table 3). Hand washing is the most efficient way to remove pathogens from the hands **(Todd *et al*., 2010; Jianu & Gole, 2014; Faour-Klingbeil *et al*., 2015)**. About 60% of respondents wash their hands before preparing fish and repeat that while working (Q3: Table 3). The majority (92%) agreed that the final product should not come into contact with any wounded hands (Q7: Table 3). While, they need to know that it is essential to pay attention to the temperature and time required to prepare the herring since 0% agreed to this information (Q6: Table 3). The percentage of the correct answer to this question came lower than the attitudes of workers in other food processing plants in China **(Akabanda *et al*., 2017)**.

Good practices were demonstrated by 52% of the workers (Table 4). About 75% (15/20 questions) of the cold-smoking fish plant workers responded correctly to more than half of the questions. Almost all workers (96%) answered positively on washing and disinfecting tools used in fish processing (Q7: Table 4). This finding agrees with workers' practices in Ghana and China **(Akabanda *et al*., 2017; Chen *et al*., 2018)**. In addition to good practices with washing hands, separation between unprocessed frozen fish and smoked herring, and excluding sick individuals from dealing with the food product. (Q1, 17 & 19: Table 4). Similarly, **Murray *et al*. (2017)** reported that, about 90% of food handlers acted upon the recommended cleaning and separating instructions when handling raw meat to prevent potentials of foodborne illness. Comparing the good attitudes and practices, the relatively low-level knowledge in this study suggests the inadequacy of the food safety training programs, suggesting further improved training on employee food-safety knowledge.

Table 2. Food safety-related knowledge among smoked-fish plants workers

Statements	N*	Correct Answers %
1) Must clean and disinfect all surfaces using liquid detergent and disinfectant.	**25**	**100**
2) Disinfectants must be stored away from food preparation areas.	**17**	**68**
3) Fish are kept in the refrigerator at 4°C for thawing and to facilitate processing.	**8**	**32**
4) It is necessary to soak the fish for a sufficient period in the brine to prevent bacteria.	**2**	**8**
5) The brine is made daily, with certain proportions and with drinking water.	**10**	**40**
6) Many types of bacteria can be present in food and cause health problems and chronic diseases.	**10**	**40**
7) Types of bacteria that cause health problems are Listeria, Salmonella and Staphylococcus.	**1**	**4**
8) Wearing gloves, masks and head coverings affect the quality of the final product.	**13**	**25**
9) It is forbidden to eat or smoke while preparing fish.	**19**	**76**
10)It is necessary to check the temperature of refrigerators/ freezers periodically to reduce the risk of food contamination	**23**	**92**
11) Infection of workers with common cold and diarrhea can cause food contamination.	**8**	**32**
12) The worker shall not work in the fish processing area and the final product packing area at the same time.	**13**	**52**
13) Tools and devices used with raw fish should not be used with processed fish.	**24**	**96**
14) Freezing does not kill all bacteria that may cause foodborne illness.	**15**	**60**
15) In the time of skin or respiratory diseases, it is necessary to stay off the job	**20**	**80**
16) Learning more about food safety through training courses is important to me.	**0**	**0**
17) Certain types of wood or smoke used in the smoking process might cause serious illnesses.	**1**	**4**
18) There is a specific way to wash hands properly.	**1**	**4**
19) Foodborne diseases affect public health.	**11**	**44**
20) Eating contaminated smoked fish may lead to miscarriage and death in pregnant women.	**25**	**100**
Poor knowledge (26-30):	**15**	**60**
Average knowledge (30-34):	**8**	**32**
Good knowledge (>34):	**2**	**8**
Score range	**26-38**	

*Number of correct respondents

Table 3. Food safety-related attitudes among smoked-fish plants workers

Statements	***No****	**Correct%**
1) I prefer wearing a head cover, a mask and a glove during the preparation of the product.	**10**	**40**
2) I prefer working in my personal clothes.	**23**	**92**
3) I like washing my hands before preparing fish and repeating that while working.	**15**	**60**
4) The fish processing area should be separated from the final product packing area.	**20**	**80**
5) I do not prefer to re-freeze fish that has been thawed for work.	**15**	**60**
6) It is not necessary to pay attention to the temperature and time required to prepare the herring.	**0**	**0**
7) The final product should not come into contact with any wounded hands.	**23**	**92**
8) It is important to wear a watch and a ring while working.	**13**	**52**
9) I like moving between different departments during the manufacturing process.	**13**	**52**
10) I prefer to inform the supervisor when any problem occurs during work or a health problem.	**22**	**88**
Negative attitude (18-21):	**3**	**12**
Neutral attitude (21-24):	**8**	**32**
Positive attitude (>24):	**14**	**56**
Score range	**18-27**	

*Number of correct respondents

Table 4. Food safety-related practices among smoked-fish plants workers

Statement	**N***	**Correct%**
1) Wash my hands before touching fresh or frozen fish.	**19**	**76**
2) Wash my hands after touching fresh or frozen fish.	**10**	**40**
3) Wash my hands before touching the final product.	**7**	**28**
4) Use gloves when touching smoked fish.	**5**	**24**
5) Frozen fish is thawed at room temperature.	**24**	**96**
6) I check the expiration date of the frozen fish upon receiving it.	**22**	**84**
7) Tools used in fish processing are washed and disinfected.	**24**	**96**
8) The fish transport boxes are washed and disinfected before and after transportation.	**13**	**52**
9) Fish transport boxes are used to transport other materials such as salt or detergents.	**14**	**56**
10) Check the temperature of the fish using a thermometer.	**0**	**0**
11) Wash the fish after defrosting.	**23**	**92**
12) Fish processing takes a long time (several hours).	**21**	**84**
13) I can eat and drink while the fish are being prepared.	**17**	**68**
14) I can smoke at any time during work.	**11**	**44**
15) Use drinking water to prepare the brine fresh daily.	**14**	**56**
16) The container in which the salting takes place is cleaned and disinfected before and after salting.	**23**	**92**
17) Unprocessed frozen fish and smoked herring are stored in the same warehouse.	**24**	**96**
18) The period of storage of the final product in the warehouse shall not exceed 3 days.	**24**	**96**
19) The supervisor shall exclude sick individuals from dealing with the final product.	**22**	**88**
20) I exclude any spoiled or damaged fish.	**22**	**88**
Incorrect practices (27-31):	**2**	**8**
Average practices (31-35):	**10**	**40**
Good Practice (>35):	**13**	**52**
Score range		**27-38**

*Number of correct respondents

Good attitudes and practices of workers in smoked fish plants reflect the safety of the final product. The total aerobic bacteria and total halophilic bacterial count of the final product were estimated as 4.17± 0.42, and 3.01± 0.71log CFU/g, respectively. These previous counts were less than the limit (<10^5 CFU/g) set by Egyptian standardization **(EOS, 2005)**. The products were free from *Staphylococcus aureus* and *Listeria* (Table 5). Still, the fungi counts were recorded as 2.77± 0.56 log CFU/g, which exceeded the standard limits. Similarly, the average APC and fungi counts were 3.16 ± 0.19, and 2.22 ± 0.18, respectively, in smoked salmon in retail markets in Egypt **(Edris *et al*., 2017)**. Final cold smoked fish products were not vacuum packed, which might expose the product to multiple contaminations, or due to improper processing during smoking **(Dutta *et al*., 2018)**.

Table 5. Bacterial count analysis (Log $_{10}$ CFU/g) of randomly selected final smoked fish products collected from the smoked-fish processing plant

Bacterial analysis	***Average count (log CFU/g)***
Total aerobic plate count	4.17 ± 0.42
Total halophilic bacteria count	3.01 ± 0.71
Total Staphylococcus count	< 1 estimated
Total Listeria count	< 1 estimated
Total yeast and molds count	2.77 ± 0.56

2. Food contact surfaces at the processing plant

Cross-contamination can result from procedures including insufficient hygiene practices, contaminated equipment surfaces, contamination by food handlers and product processing **(Carrasco *et al*., 2012; Jones *et al*., 2020)**. Total aerobic bacterial counts (APC) in this study ranged from 4.33 ± 0.76 logs in smoking hooks to 6.13 ± 0.32 logs in the frozen fish reception table. Total coliform count (TCC) as a hygienic indicator was relatively high on almost all swabbed surfaces. TCC reaches up to 5.53 logs in the frozen fish reception table (Table 6). The levels of the two different microbial indicators APC and TCC were reported from the food contact surface of the catfish industry in the United States **(Abdallah-Ruiz *et al*., 2022)**. APC was up to 4.8-4.9 logs at fish de-header and skinner, while the max count of 2 logs of TCC was associated with whole fish skinner, holding tray and manual fish conveyor. APC levels were recorded above the microbiological limits (APC < 2.4 logs CFU/100 cm^2) (**Henroid *et al*., 2004)**. The elevated count of APC on some surfaces might suggest the presence of an inadequate cleaning of organic matter during processing.

Table 6. Bacterial count (Log $_{10}$ CFU/100 Cm^2) of different swabbed food contact surfaces in the smoked-fish processing plant

Food contact surface	APC	*Salmo--nella* (+/-)	Total Coliform count	Total *Staph. aureus* count	Total *Listeria* count
Raw fish transport boxes	5.5 ± 0.5[ab]	+	4.16 ± 0.57[cd]	NT	NT*
Balances for raw fish	5.77 ± 0.21[ab]	+	3.73 ± 0.81[d]	1.71 ± 0.22[c]	NT
Frozen fish reception table	6.13 ± 0.32[a]	+	5.53 ± 0.35[a]	NT	NT
Hand washing faucet	5.8 ± 0.27[ab]	-	1± 0.00[g]	3.22 ± 0.55[a]	6.00± 0.5[a]
Salt barrel (Inside surface)	5 ± 0.5[bc]	-	1.82 ± 0.43[fg]	NT	NT
Hooks used to dry fish	5.83 ± 0.21[ab]	-	4.17 ± 0.29[cd]	2.53 ± 0.065[b]	NT
Smoking room	5.8 ± 0.27[ab]	-	4.67 ± 0.29[bc]	2.34 ± 0.55[b]	NT
Smoking hooks	4.33 ± 0.76[c]	-	1.90 ± 0.51[f]	3.29 ± 0.36[a]	NT
Final product weighing table	5.5 ± 0.5[ab]	-	2.69 ± 0.70[e]	1.86 ± 0.38[c]	5.5 ± 1.00[a]
Vacuum device	5 ± 0.5[bc]	-	4.37 ± 0.32[cd]	NT	NT
Freezing chamber (-35°C)	2.5 ± 0.5[c]	-	1±0.00[g]	NT	5.5 ± 1.00[a]

*Not tested; APC: Aerobic plate count

This study identified *Salmonella* in different areas that come in contact with raw fish such as transport boxes, balances and reception tables (Table 6). Similarly, three isolates of *Salmonella* were identified from the food contact surface of the seafood processing plant. This was attributed to the ability of *Salmonella* to form biofilms on different contact surfaces **(Surya *et al.*, 2023)**. Moreover, the total *S. aureus* and total *Listeria* count have an impact on this outcome. Although the attachment strength of *Listeria* spp. to contact surfaces was not done during this study, others described that it can attach better to plastic than to glass and stainless steel **(Veluz *et al.*, 2012)**. Therefore, it is important to have an effective removal of organic waste and cleaning before the application of sanitizers on those sites. In addition, biofilms are very different to remove and can harbor *L. monocytogenes* or *Salmonella,* making it more difficult to remove this pathogen from processing plants (**Abdallah-Ruiz *et al*., 2022)**. The cleaning procedures for food-contact-surfaces should be evaluated, and special care should be given to all utensils used during receiving raw fish and during processing.

3. ASC application and ATP bioluminescence assay

The bioluminescent system measures the presence of ATP in a sample uses luciferin-luciferase enzyme system **(Lappalainen *et al*., 2000)**. The total light output of the sample is directionally relative to the quantity of ATP present in the sample **(Mandal *et al.*, 2011)**. ATP measuring devices are used to monitor the efficacy of a sanitation system within the food industry **(Lappalainen *et al*., 2000)**. ATP is a useful indicator for total microbial loads on an environmental surface but is not a valid measure of pathogenic microorganisms in a food processing facility **(Osimani *et al*., 2014;**

Hammons *et al*., 2015). The total bacterial count estimated using ATP level was shown in Table (7). The application of acidified sodium chlorite (ASC) was better in decreasing the bacterial count for less than 1 log reduction compared to hot water, and tape water as a control on food contact surfaces. ASC is usually reported to be applied directly to food such as vegetables and fish **(Su *et al*., 2003; Inatsu *et al.*, 2010; Zhou *et al*., 2020)**. As a new approach, the results of this work indicated its efficacy on food-contact surfaces of stainless-steel tables, cutting plastic boards and knife steel blades. The concentration of 1500 ppm of ASC produces the best reduction in total bacterial count compared to 500, and 1000 ppm concentrations, without significant difference (p=0.07). **Surya *et al*. (2023)** reported a reduction of 2-3 log CFU/100 cm^2 on surfaces of stainless-steel seafood contact surfaces when treated with a minimum of 20mg/ l of sodium hypochlorite after a contact time of 5min.

Table 7. the use of ATP bioluminescence assay swabs to indicate the total bacterial count (log CFU/100 Cm^2) after the application of acidified sodium chlorite sanitizer

	500ppm			***1000ppm***			***1500ppm***		
	tap water	**hot water**	**sanitizer**	**tap water**	**hot water**	**sanitizer**	**tap water**	**hot water**	**sanitizer**
Stainless steel table	3.43 ± 2.59	3.22 ± 2.46	2.92 ± 1.72	3.20 ± 2.47	2.95 ± 2.13	2.48 ± 2.12	3.18 ± 2.96	3.01± 2.76	2.58 ± 2.10
Cutting board	3.51 ± 3.27	3.35 ± 2.83	2.98 ± 1.75	2.58 ± 3.11	3.38 ± 2.85	2.96 ± 1.57	3.64 ± 3.02	3.31± 1.38	2.73 ± 1.80
Knife	2.85 ± 1.63	2.77 ± 2.00	2.31 ± 2.12	2.75 ± 1.85	2.55 ± 1.80	1.99 ± 0.75	2.77 ± 1.41	2.51 ± 1.57	1.91 ± 0.85
Smoked fish	4.95± 0.05	NT*	4.34 ± 0.47	4.95± 0.05	NT	4.04± 0.90	NT	NT	NT

*Not tested

When ASC was applied directly to cold smoked fish, it successfully reduced the total bacterial count. The bacterial count was reduced to 4.34 and 4.04 logs CFU/g for 500 and 1000 ppm concentrations, respectively, compared to the control (4.95 CFU/ g). No significant differences (P>0.05) were estimated in the bacterial reduction applied to smoked fish between 500 and 1000 ppm ASC concentrations. The total count of bacteria after the application of ASC was estimated using the agar plate count method. Similarly, 50 ppm ASC applied on salmon fillets for 1min resulted in *L. monocytogenes* reduction of 0.5 log CFU/g **(Su *et al*., 2003)**. Besides, no significant differences were detected between 50 ppm and 1000 ppm ASC when it was used as an antimicrobial treatment for rainbow trout fillets **(Kamireddy *et al*., 2008)**.

CONCLUSION

- Comparing the good attitudes and practices, the relatively low level knowledge presented in the study suggests the inefficiency of the food safety training programs. Further improved training is suggested on employee food safety knowledge. Good attitudes and practices of workers in smoked fish plants reflect the safety of the final product. The total aerobic bacterial count (APC) and total halophilic bacterial count of the final product were estimated as 4.17± 0.42, and 3.01± 0.71 log CFU/g, respectively. APC was less than the standard limit, along with the products being free from *Staphylococcus aureus* and *Listeria*.
- APC and total coliform count as hygienic indicators were relatively high on almost all swabbed surfaces. *Salmonella* was identified in different areas that come in contact with raw fish as well as high counts of *Listeria* and *Staphylococcus*. The concentration of 1500 ppm of ASC produces the best reduction on surfaces in APC, compared to 500 and 1000 ppm concentrations. When ASC (500, 1000 ppm) was directly applied on cold smoked fish, the APC was reduced from 4.95 to 4.34 and 4.04 logs CFU/g, respectively.
- Good knowledge of workers and adequate cleaning procedures with the application of ASC (500, 1000, and 1500 ppm) on food contact surfaces and smoked fish are needed to improve food safety in cold-smoking fish plants in Egypt.

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