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COMPARATIVE EFFICACY OF CHLORINATED AND NON-CHLORINATED DISINFECTANTS ON FOOD CONTACT SURFACES IN A TUNA PROCESSING AREA

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ABSTRACT

The use of disinfectants is indispensable in food processing industries. The ability of these chemical products to satisfactorily reduce the microbial load on food contact surfaces is fundamental in maintaining proper sanitary conditions and enabling the production of safe food. This study compares the disinfectant efficacy of chlorine (SuperChlor), peroxyacetic acid (OxyPlus) and a quaternary ammonium based agent (BacPlus) on food contact surfaces in a tuna processing area. A master sanitation schedule was implemented at the end of each week whereby fish processing tables were manually washed with alkaline detergents Multi-Kleen and subsequently foamed with Power-Foam (5%). After proper rinsing, surfaces were treated with disinfectants SuperChlor (25ppm), Oxyplus (1%) or BacPlus(1%). The surfaces were swabbed before and after treatment. Cleaning followed by disinfection with BacPlus, SuperChlor or OxyPlus were found to reduce the total viable counts by 3.4, 2.5 and 2.5 log/cfu/cm^2 respectively although no significant differences were observed among the three tested products (P>0.05). This study indicates that quaternary-ammonium based disinfectant has a higher bactericidal efficacy than other tested products when applied in a fish processing area and suggests that it can be used in conjunction with or as an alternative to existing products.

Keywords: **Disinfectants**, **Food Processing, Tuna, Mauritius**

INTRODUCTION

The fisheries industry is of great importance in Mauritius. A great amount of revenue is generated from export of seafood, mainly of tuna processed products. The main markets for exports are Spain, UK and the US. These countries have been constantly upgrading and enforcing their requirements for implementation of Food Safety Management Systems and Quality Management Systems. These include implementation of HACCP, ISO 9001:2008 and ISO 22000:2005 or the British Retail Consortium to which countries such as Mauritius have to abide to. However, all these standards are based on Pre-Requisite Programs (PRPs), which are essential for sustainability of any food industry. One important, albeit basic PRP, is cleaning and disinfection. Cleaning and sanitation of food contact surfaces is a foremost activity in food industries to prevent cross-contamination of both ready-to-eat foods and ready-to-process foods *(19)*. Usually, food manufacturers have to abide by the clients' hygiene requirements and extending processing hours to increase the production tonnage can inadvertently reduce time dedicated to cleaning and disinfection activities *(8)*. The development of a Sanitation Standard Operation Procedure (SSOP) is essential to increase the effectiveness of a cleaning procedure. However, an important consideration of SSOPs relies on the choice of appropriate disinfectants and proper method of application to ensure a satisfactory reduction of microbial load from food contact surfaces *(8)*. Disinfectants have been identified as a last line of defence against pathogens in a manufacturing industry *(14)*. Sanitation in food industries is thus of prime importance as inadequacies in cleaning and disinfection can be a cause for transmission of food borne diseases by cross-contamination and this risk is amplified when food is traded among countries *(7)*.

Detergents are cleaning agents which aim at removing soilage (food debris, grease and fats, proteins) from food contact surfaces and enhance subsequent disinfection. Disinfectants can have different physicochemical characteristics and should be carefully chosen for the type of soilage to be removed from a surface. An understanding of the properties of different types of sanitizers or disinfectants with respect to their antimicrobial spectrum, contact time, temperature and concentration of application is essential to effectively reduce the microbial load on food contact surfaces *(2)*. It is assumed that a proper cleaning and disinfection procedure should be able to decrease high microbial load of 7-8 log/cm^2 to low levels of 1 log/cm^2*(20)*. However, due to the continuous use of a single sanitizer, bacteria may develop resistance over time and inherently resistant microbes present on the surfaces can multiply *(18)*. Hence a sound sanitation schedule requires disinfection with alternative products *(18)* having different mechanisms of action. Various sanitizers exist and they are either chlorine-, iodo phore-, peroxy acetic acid-or quaternary ammonium-based and should be able to reduce microorganisms to a minimum of 1 log/cm^2*(20)*.

The objective of the study was to evaluate the disinfection efficacy of one chlorinated disinfectant; SuperChlor and two non-chlorinated disinfectants; Oxyplus (peroxyacetic acid based) and Bacplus (quaternary ammonium based) in a tuna processing plant which is a major exporter of frozen cooked tuna loins to various European countries and the U.S.

MATERIALS AND METHODS

Study area

This study was carried out in the "fish cleaning section" of the plant where cooked tuna loins are manually pre-cleaned and cleaned. This area comprised of 5 processing tables of which Tables 2 and 4 were used for the study as shown in Figure 1.

Chemical detergents and disinfectants

The detergents and disinfectants compared in this study and their respective characteristics or active ingredients are outlined in Table 1. The order in which the chemicals were applied is summarized in Figure 2.

Procedure for cleaning and disinfection of tables

Prior to the start of cleaning, all trays used to hold cooked tuna were removed from the processing lines and sent for washing. High-pressure water jets were applied to remove any tuna debris that remained on the processing tables at the end of the previous shift. Alkaline detergent Multi-Kleen was first applied manually onto the tables and these were scrubbed with the help of scrubbing sponges. High-pressure water jets were then used to rinse the processing tables several times before the application of the alkaline detergent Power-Foam using a foam generator. Tables were again rinsed with High-pressure water jets. After proper rinsing of the surfaces, the disinfectants were then applied. Finally, the surfaces were rinsed with water and wiped with wipers. The surface doses, contact time and mode of application of the chemicals are summarized in Table 2.

Microbiological sampling and analysis

The four extremities of Tables 2 and 4 [NR (Near Right), NL(Near Left), FR(Far Right) and NR(Near Right)]- were sampled as indicated in Figure 1. For this purpose, transportable swabs pre-moistened in 0.1% buffered peptone water were used to swab a surface of 25cm^2 (5cm x 5 cm) by gently rolling the swab in a zig-zag motion as indicated in Figure 3*(10)*. The swabs were then transported to the laboratory in a cooler box and were microbiologically analyzed for recovery of the microorganisms. Briefly, swab samples were rinsed in 0.1% of Buffered Peptone water (Oxoid) by vortexing for at least 10 seconds and the rinsate analyzed for Total Viable Count on Plate Count agar*(13)*, *Escherichia coli* on Eosin Methylene Blue agar *(4)* and *Listeria monocytogenes* on PALCAM agar *(11)*.

Statistical analysis

All experiments were conducted in two independent trials. Microbial counts were log transformed and statistical analyses were conducted using Minitab® Release 17. A single factor analysis of variance (ANOVA) and Tukey's one-way multiple comparisons were conducted on the disinfectant efficacy (DE) of each chemical where DE = [Total viable count before treatment (log cfu/cm^2)] – [Total viable count after treatment (log cfu/cm^2)]. Significant differences were considered at the 95% confidence level ($P < 0.05$).

To determine whether each disinfectant was able to significantly reduce the microbial load after treatment, a paired t-test (α=5%) was carried out to compare the total viable counts (log cfu/cm^2) before and after treatments.

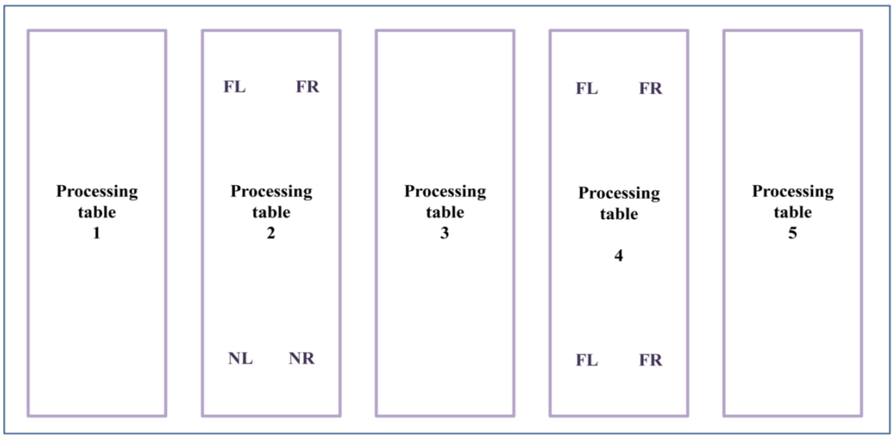

Figure 1: Layout of the "fish cleaning section" of the production floor comprising of 5 stainless steel tables dedicated to cleaning of cooked tuna loins. Tables 2 and 4 were chosen for the study

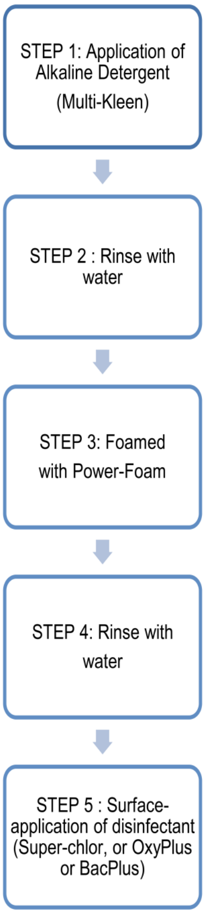

Figure 2: Sequence of steps for cleaning and disinfection procedure

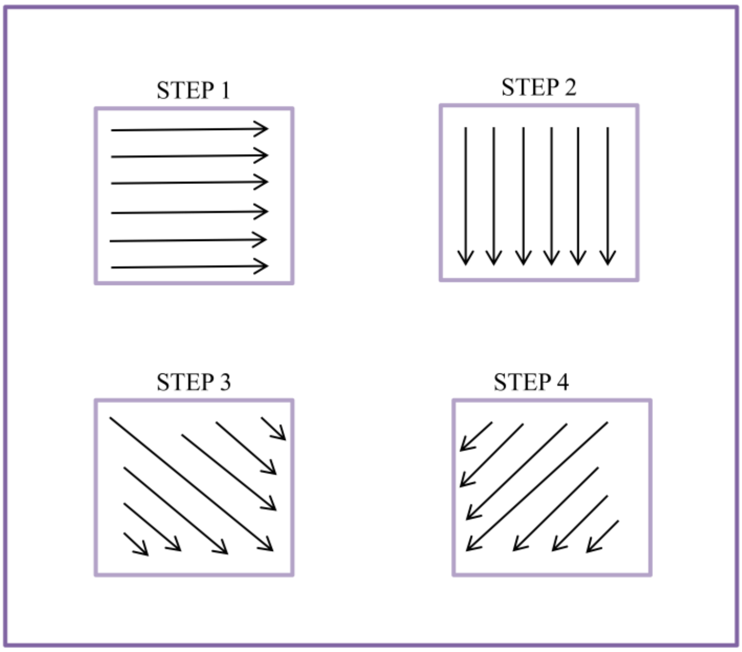

Figure 3: Series of steps for swabbing

Table 1: Chemicals and their nature

Function of chemicals	Commercial Name	Nature
Detergents	Multi-Kleen	Foaming and alkaline
	Power-Foam	Self-foaming and alkaline
Disinfectants	SuperChlor	Calcium hypochlorite
	OxyPlus	Peroxyacetic acid
	BacPlus	Quaternary ammonium compound

Table 2: Dosage, contact time and application method of each chemical

Chemical name	Concentration (%)	Time contact (min)	Method of application
Multi-Kleen	Pure concentrate	10	Manual scrubbing
Power-Foam	5	10	Foam generator
SuperChlor	0.0025	10	Spreading
BacPlus	1	10	Spreading and rinsing with water
OxyPlus	1	10	Spraying and rinsing with water

RESULTS AND DISUSSION

Compliance of Total Viable Count to Company and EU limits

Total Viable Count is an indicator of surface hygiene and an increase in its counts is suggestive of hygiene failures in a food safety management system *(17)*. At each location sampled, all three disinfectants were able to reduce TVC with a mean initial load of 3.6 – 4.0 log cfu/cm^{2}toa final mean level of 0.7 – 1.2 log cfu/cm^2 (Table 3), which is in compliance with the maximum acceptable internal limit set by the company of <2 log cfu/cm^2. However, SuperChlor and OxyPlus were unable to meet the 2001/471/EC limit of < 1 log/cm^2, unlike BacPlus, which was able to reduce the microbial charge to a mean final count of 0.7 log cfu/cm^2. Therefore SuperChlor and OxyPlus may not be suitable for use alone since they do not meet the required 2001/471/EC standard. Alternatively, these disinfectants could be used in tandem or in conjunction with other chemicals to reduce the microbial burden to the acceptable final level. The lower antibacterial efficacy of chlorine-based disinfectant could be attributed tothe progressive disappearance of free available chlorine in the presence of organic chlorine demand substances *(21)*.Chlorine can react rapidly with natural organic matter (NOM) at varying rates to form a range of organo-chloride compounds, some of which may pose a potential threat to the health of consumers *(12)*.Chlorine can also react with inorganic substances in a reduced valence state such as iron, manganese, ammonia and bromide. Experiments demonstrated that a mixture of 2.71% sodium hypochlorite and 7% sodium hydroxide, diluted to 3-4% and applied for 15-20 minutes reduced total aerobic plate count by at most 2.1 log cfu/cm^2 respectively and therefore the chlorine-containing disinfectant was unable to meet the 2001/471/EC requirements as concluded by the author *(5)*. It is also worth mentioning that there were no significant differences in the residual TVC post-disinfection at the four table extremities (Table 5). This is indicative of the robustness of the tested products in delivering consistent levels of bactericidal activity despite the existence of micro differences in surface roughness, hardness, wettability and finish that can be present on stainless steel surfaces *(1)*.

Escherichia coli

All 3 products were able to reduce *E. coli* present at a mean initial level of 1 log cfu/cm^2 to undetectable levels after disinfection (< 0.1 log cfu/cm^2). Similarly, undetectable *E. coli* counts were noted after cleaning and disinfection with chlorine *(3)*.All three products had a satisfactory colicidal efficacy despite their different chemical properties (oxidizing vs. non-oxidizing agents), mechanisms of action and cellular targets. Chlorine being a potent oxidizing agent oxidizes enzymes, inhibits protein synthesis, decreases ATP production, breaks in DNA and depresses DNA synthesis while peroxyacetic acid and quaternary ammonium compounds interfere with the functioning of the cell wall and cell membrane respectively *(6)*.

Listeria monocytogenes

L. monocytogenes was neither found before nor after cleaning and disinfection. Similarly, in a study on the effectiveness of two sanitation procedures in a dessert processing factory, analyses for *L. monocytogenes* using a microbiological kit (Path-Check Hygiene Pathogen System) were negative after disinfection of food contact surfaces, although it was detected on non-food contact surfaces especially on floors *(5)*.However, in a study carried out in a salmon processing plant, a prevalence rate of 16% for *L. monocytogenes* was noted *(22)*. Additionally, listeriae were found in different food processing units at a variable detection rate suggesting their sporadic presence *(16)*.

Disinfectant Efficacy (DE) at each sampling location

The Disinfectant Efficacy (DE) gives a measure of the log reduction of Total Viable Count achieved after cleaning and disinfection. All three disinfectants could significantly reduce TVC on the tested tables after cleaning and disinfection ($p < 0.05$) although they could not achieve complete elimination (Tables 4 and 5). Mean reductions of 2.5, 2.5 and 3.4 log cfu/cm^2 (Tables 4 and 5) were achieved for

SuperChlor, OxyPlus and BacPlus respectively. BacPlus was more effective than OxyPlus and SuperChlor at reducing the microbial load from the processing tables although there was no significant difference in their DE (p>0.05). In addition, there were no significant differences in the DE of the three products among the four table extremities (Table 5). It has previously been reported that peroxyacetic acid and quaternary ammonium compounds were more bactericidal than hypochlorite-based chemicals, especially in the presence of fat*(8)*. Indeed, one of the most important functional properties of a disinfectant is its ability to remain active even in the presence of organic matter and compatibility with soaps, detergents, and other chemicals encountered in use *(6)*.

It is also worth noting that the contact time for each disinfectant tested was maintained at 10 minutes as recommended by the chemical manufacturer. For instance, general-purpose disinfectants were demonstrated to achieve a 3-log reduction of surface-adherent cells if sufficient time of contact was allowed *(9)*. However, SuperChlor and OxyPlus were unable to achieve ≥3log reduction cycles despite a contact time of 10 minutes. We attribute it to the possible presence of interfering substances. As reported elsewhere, disinfectants can bind to soils thereby affecting the degree of reduction of microbial load*(15)*.In a study evaluating the efficacy of chlorine, it was concluded that organic matter present on food contact surface scan interfere with chlorine thereby reducing its bactericidal efficacy *(23)*. During visual assessment, fish debris and grease were observed on food contact surfaces after cleaning and disinfection and their presence could have influenced the efficacy of the disinfectant. On the contrary, the antimicrobial activity of BacPlus is not generally affected by the presence of organic matter *(8)*.

Table 3: Mean Total Viable Counts recovered from surfaces before and after treatments with different disinfectants

Disinfectant	Mean Total Viable Counts (log cfu/cm^2)	
	Before	After
BacPlus	3.9 ± 0.61^a	0.5 ± 0.78^b
OxyPlus	4.0 ± 0.68^a	1.5 ± 0.88^b
SuperChlor	3.6 ± 0.91^a	1.1 ± 0.62^b

Values in the same row followed by the same superscript letters were not significantly different (P > 0.05).

Table 4: Residual Total Viable Counts (log cfu/cm^2) recovered from each table surface extremity after disinfection

Table Extremity	Super-Chlor	Oxy-Plus	Bac-Plus
Near left	1.5 ± 0.75	1.1± 0.88	0.3± 0.11
Near right	0.9 ± 0.31	1.9± 0.86	0.9± 0.66
Far left	1.2 ± 0.56	1.5± 0.9	0.6± 0.68
Far right	1.2 ± 0.40	1.6± 0.59	0.4± 0.19

Values in the same column for each disinfectant were not significantly different (P > 0.05). Values in the same row for each table extremity were not significantly different (P > 0.05)

Table 5: Comparison of DE (log cfu/cm^2) of SuperChlor, OxyPlus and BacPlus at different table surface extremities

Location	DE (log cfu/cm^2)		
	Super-Chlor	Oxy-Plus	Bac-Plus
Near left	2.4 ± 0.97^a	2.7 ± 0.20^a	4.2 ± 0.72^a
Near right	2.8 ± 1.61^a	2.7 ± 0.71^a	2.6 ± 1.30^a
Far left	2.5 ± 1.10^a	2.4 ± 0.73^a	3.3 ± 0.81^a
Far right	2.1 ± 0.85^a	2.1 ± 1.09^a	3.5 ± 0.75^a
Mean	2.5 ± 1.07	2.5 ± 0.75	3.4 ± 1.03

Values in the same row followed by the same superscript letters were not significantly different (P > 0.05).

CONCLUSIONS

This study compared the bactericidal efficacy of chlorinated and non-chlorinated disinfectants included in the sanitation program of a tuna processing plant. Chlorine was more frequently used than two other non-chlorinated disinfectants (peroxyacetic acid and quaternary ammonium) by virtue of its relatively low cost, wide availability and ease of application. Findings from this study indicated the limited efficacy of oxidizing agents such as chlorine and peroxyacetic acid compared to non-oxidizing quaternary ammonium-based compound. Moreover, only quaternary ammonium-based product BacPlus could meet the regulations imposed by importing countries governing general hygiene requirements for food premises. Overall, these results point to the need to regularly review the validity of the sanitation program, the choice of disinfectants used and the need to consider using additional or alternative agents to meet these limits.

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