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THE SANITISING EFFICIENCY OF DIFFERENT DISINFECTANTS USED IN THE FISH INDUSTRY

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ABSTRACT

This study examines the bactericidal efficiency of common disinfectants using the modified surface testing method against adherent cells on stainless steel surface (type 304, 2B). Mixed culture of *Pseudomonas putida*, *Serratia liquefaciens* and *Shewanella putrefaciens* isolated from shrimp and fish processing plants were used as test bacteria. Cod juice (*Gadus morhua*) and Atlantic herring juice (*Clupea harengus*) were used to simulate the practice processing condition of lean fish and fat fish. The results from this project indicated that adhesion of selected bacteria suspension on stainless steel was weak and adherent cells could be removed easily by running water. At concentrations of 50 and 200ppm of active chlorine hypochlorite containing disinfectant was less effective than peracetic acid (PAA) and quaternary ammonium compound (QAC) at concentrations of 0.25, 0.5 and 1.0% (v/v) especially in the presence of fat. The type of raw material produced must be considered as being a predominating parameter affecting the time-concentration relation of the applied disinfectants.

Key words: disinfectant; hypochlorite; peracetic acid; quaternary ammonium compound; lean; fat.

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1 INTRODUCTION

During the past decade, production of food has become more complex; the production volumes are larger, the operations are more mechanical, the food is more processed and the time and distance between production and consumption are longer. The new trends in food production and consumption lead to an increased need for efficient sanitary practices in the food processing industry regarding the EU's new stringent safety requirements on hygiene, i.e. Regulation (EC) No. 852/2004, Directive 2004/41/EC, that were adopted in 2004 and will take effect on January 1st, 2006 (Arvanitoyannis *et al*. 2005).

According to the new regulations and directives on hygiene, sanitary conditions in food processing plants are required to be at a high level. To meet these requirements an effective disinfectant should be used, and an appropriate concentration of this disinfectant should be applied in well-defined application.

Disinfection is the final stage in a sanitation programme that needs to be designed perfectly to ensure both the safety and quality of food (Schmidt 2003). However, due to lack of information it is not easy to perform disinfection properly. In fact, how to perform disinfection properly remains a matter of concern. Despite quite widespread use of disinfectants in fishery industries, the recommended in-use concentration of disinfectant is often based on laboratory suspension tests. They usually do not reflect the real application conditions of disinfectant and the range of bacteria tested may be too limited. Consequently, disinfection sometimes does not eliminate the bacteria to the extent expected which may result in critical hazards to the health of consumers. Therefore, more studies into the bactericidal properties of disinfectants at different concentrations, contact times and presence of organic matters are required to establish guidelines for their application.

Chlorination is widely practiced for microbial control in fishery plants in Vietnam due to its low cost and high bactericidal efficiency. However, several drawbacks of chlorine-based disinfection systems have been identified, including the formation of (potentially) hazardous disinfection by-products and the discovery of water-borne microbial pathogens that are relatively resistance to chlorine (Greene *et al.* 1993). These factors require not only scientists but also fisheries producers to take into consideration the application of alternative disinfectants to chlorine-containing disinfectants.

Increased knowledge and better understanding of the bactericidal capacity of disinfectants are essential to optimise sanitation procedures, as well as, to reduce costs, environment waste and to improve shelf life. This study will evaluate bactericidal efficiency of alternative disinfectants to chlorine in conditions of using fish juices of cod (*Gadus morhua*) and Atlantic herring (*Clupea harengus*) to simulate the environments processing lean and fat fish. It is also good preparation for more intensive research regarding both disinfection and hygiene in Vietnam.

2 OBJECTIVES

The aim of this study was to evaluate bactericidal efficiencies of some commercial disinfectants. The efficiency of three disinfectants was tested against bacteria isolated from fishery processing environments, *Pseudomonas putida, Serratia liquefaciens*, and *Shewenlla putrefaciens*, adhered to stainless steel AISI 304, the most frequently used material for food processing surfaces (Rossoni *et al*. 2000). The efficiency of the disinfectants was determined by the calculating the difference between the number of microorganism remaining on the stainless steel coupon before and after being treated with the disinfectants. The effects of several parameters such as time, concentration, and interfering substances on disinfection were discussed.

3 LITERATURE REVIEW

3.1 Hygiene and disinfection in general

All fish industries have to adhere to the food hygiene directives to ensure that products have been produced according to existing laws recognised processing methods and hygiene standards. Therefore, chemical disinfectants are commonly used in the seafood industry against harmful microorganisms on surfaces coming into contact with food to improve food hygiene. Although food producers usually use excess levels of disinfectant typically 20% above the recommended dosage (Moody *et al.* 2001), the application of chemical disinfectants in food industries has been shown to be inadequate in terms of total aerobic heterotrophic bacteria counts on the food contact surfaces (Taylor *et al*. 1999, Miettinen *et al*. 2001). This was also shown by Bagge-Ravn *et al*. (2003b) with their study performed in four plants producing cold-smoked salmon and semi-preserved herring products. The results from Bagge-Ravn *et al*. (2003b) demonstrated that microorganisms could be detected on the processing equipment after cleaning and disinfecting procedures were applied. All of these findings indicate that to achieve effective disinfection in fish plants is not easy and dependent on expert knowledge and experience (Bredholt *et al.* 1999).

3.2 Bacteria in a fishery processing environment

Bacteria are one of the main sources that have caused food-borne diseases and spoilage of products (Chmielewski and Frank 2003). A large number of studies have unequivocally demonstrated that processing equipment can be a source of bacterial contamination of food products (i.e. Blackman and Frank 1996, Bagge-Ravn *et al.* 2003b, Kusumaninggrum *et al.* 2003). Microbial contamination on environmental surfaces may be transferred to the food products directly through surface contact or by vectors such as personnel, pests, air movement or cleaning regimes (Blackman and Frank 1996, Miettinen *et al*. 2001). Bacteria, therefore, need to be removed properly from the processing environment by sanitation programs.

Although knowledge of the adhering microflora is essential in the Good Hygienic Practices programme of food processing plants, very little has been known about the more general microbiology of food processing surfaces in contrast to the specific pathogens such as *Listeria monocytogenes* (Bagge-Ravn *et al*. 2003b). The studies of Bagge-Ravn *et al*. (2003b) in four different fish processing plants as mentioned above showed that the flora was a mixture of many species and they were different in each fish processing plant. The results also demonstrated that microflora was partly a reflection of the fish processed and partly a reflection of the preservation parameters used in the products. Simultaneously a high proportion of Gram-negative was detected in fish processing plants during production, ranging from 56 to 70% of total bacteria. These findings are in agreement with a study by Guðbjörnsdóttir *et al*. (2005) in fish fillets and cooked shrimp processing plants where the predominant genus attached to the food contact surfaces were *Pseudomonas* spp. (19%) and *Enterobacteriacea* (27%); the main species were *Serratia liquefaciens*, *P. fluorescens* and *P. putida*. In addition, the results of Bagge-Ravn (2003b) showed that the majority of bacteria that remained on the surface after cleaning and disinfection were Gram-negative bacteria (from 47 to 90% of the total residual bacteria). These findings noted that Gram-negative bacteria were predominant in fish processing plants and they were better at adhering to surfaces, more resistant to disinfectant and could survive without nutrients.

3.3 Hygiene monitoring methods

Hygiene monitoring methods that detect microorganisms and food residues on product contact surfaces provide a direct and relevant measurement of cleaning efficiency and hygiene (Easter 2003). The main hygiene monitoring methods used commonly in fish industries are as follows:

3.3.1 Traditional hygiene monitoring

The oldest and most widely used method for monitoring hygiene is swabbing either with sterile swabs or sponges, rinsing, and cultivating the collected bacteria (Miettinen *et al*. 2001). These methods provide information about the concentration of microbes present on the surface and also have the advantage of being able to detect specific indicator organisms and to examine hygiene conditions in the places where space is limited (Huss 2003). However the results are generally available in 24-72 hours, which is too slow to provide usual feedback information to the sanitation and manufacturing processes and ensure that high standards of food safety and quality are maintained (Easter 2003). In addition, significant factors have been identified that influence enumeration results such as the plating method, the mode of medium preparation, the contact time temperature, the type of culture medium and the medium manufacturer (Augustin and Carlier 2006). The detection limitation of the method is dependent on the dilution used (Downes and Ido 2001).

3.3.2 Alternative methods and techniques

Several alternative methods for measuring the hygiene status of product contact surfaces that give rapid results to facilitate immediate corrective action have been developed, and some of them are simple enough to be performed on the production floor without needing a laboratory (Easter 2003). Alternative methods can be divided into the following categories: bioluminescence, protein tests and cell counting methods.

Bioluminescence:

The principle of this technique is based on using the enzyme and substrate of the firefly (luciferase and lucifein) to estimate ATP, the basic energy currency molecule of all types of living organisms. Total ATP collected by swabbing a surface is related to the amount of residual food and microorganisms. Therefore it reflects the sanitation conditions (de Boer and Beumer 1999). ATP bioluminescence is a rapid biochemical method. The rapid response time for obtaining results, ranging from seconds up to a few minutes, made this system very suitable for on-line monitoring (Huss 2003) and it has been applied increasingly in fish plants. Although there are several commercial systems for measuring ATP bioluminescence and hygiene applications, all of them have some limitations since the outcome is influenced by pH and the detection of low levels of microorganisms is not possible (de Boer and Beumer 1999).

Protein test:

In this technique protein concentration is used as a marker of surface contamination remaining after cleaning operations. The protein collected by swabbing will react with a chemical agent and result in a visible colour change depending on the level of protein. The degree of colour change is compared to a supplied colour card as an indication of the hygienic nature of the surface. Although protein tests are rapid and cheap, their applicability is less widespread than ATP tests as they were not developed to exploit the relationship between protein concentration and microorganism numbers and furthermore it does not detect fat residues (Holah 1999).

Contact plate

Contact plate is a method counting cells directly, usually known as RODAC-plate. The procedure of this method is rather simple and can be described as follows. Petri dishes or contact slices with selective or general purpose agar media are stamped to the tested surface and then the plates with adherent cells are incubated at ambient temperature and the number of the colony forming units is evaluated after 24-48 hours (Huss 2003). This method is limited with heavy contaminated surfaces and it is not applicable to porous and unsmooth surfaces (Jay 1992).

3.4 Disinfectant usages

The focus on safer foods and longer shelf-life has led to more frequent use of chemical disinfectants (Langsrud *et al*. 2003a). The major classes of the disinfectants are iodophors, quaternary ammonium compounds (QACs), peroxy compounds, chlorine compounds, acid-anionic, phenols, ozone and carboxylic acid (Huss 2003). Below is a brief description of some of disinfectants that are commonly used in seafood plants including chlorine, peroxygens and quaternary ammonium compounds.

3.4.1 Chlorine compounds

Chlorine and products that produce chlorine comprise the largest and most common group of food plant disinfecting agents due to its low cost, ease of application, and ability to inactivate a wide variety of microorganisms. Commonly used chlorine compounds include: liquid chlorine, hypochlorite, inorganic chloramines and organic chloramines (Schmidt 2003). Chlorine exits in more than one chemical state when dissolved in water and hypochlorous acid is the most effective chemical form of chlorine (Ritenour 2002). Although chlorine works well at cold temperatures and tolerates hard water, the effectiveness of chlorine is reduced if the pH of solutions is elevated as well as if organic soling matters are present. At low pH levels, bactericidal efficiency of these disinfectants is very unstable (Huss 2003). The most significant disadvantages of chlorine are that they can be corrosive to equipment and pose health risks to humans and wildlife due to the formation of undesirable halogenated compounds, such as trihalomethanes, haloacids, haloacetonitriles and other carcinogenic halo-organic compounds (Fiessinger 1985). Because of the toxicity of these disinfection by-products, optimising the chlorine application and seeking alternatives to chlorinated sanitising agents are important to reduce the release of chlorinated chemical residues to the environment (Greene *et al.* 1993).

3.4.2 Quaternary ammonium compounds

Quaternary ammonium compounds (QACs) are a class of compounds, which have the general structure as shown in Figure 1. The properties of these compounds depend upon the covalently bound alkyk groups (R-groups), which can be highly diverse (Schmidt 2003).

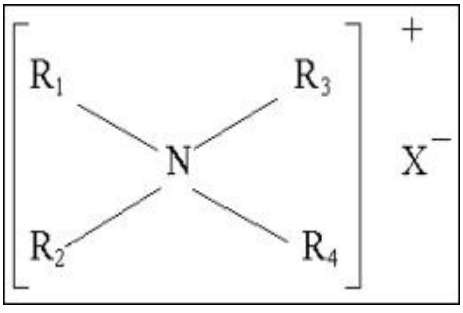

Figure 1: The general structure of quaternary ammonium compounds (Schmidt 2003).

QACs are widely used in disinfection operations in food processing industries because they have several advantages over other commonly used disinfectants (Langsrud and Sundheim 1997). QACs are cationic surfactant sanitizers and also have some cleaning activity (Schmidt 2003). QACs are effective against molds, yeast (Carsberg 1996), Gram-positive and Gram-negative bacteria except *Pseudomonas* spp., a dominant bacteria in the seafood processing environment (Langsrud *et al*. 2003b). QACs are non-corrosive, non-irritating, and their activity is unaffected by organic load. Under recommended usage and precautions, QACs pose little toxicity or safety risks (Schmidt 2003). They require a relatively long contact time to achieve significant kill and are therefore often applied as foam (National Seafood HACCP Alliance 2000). However, the broad application of QACs in food industries can cause the possibility of microbial growth and adaptation (Sundheim *et al*. 1998, Langsrud *et al*. 2003b). To reduce the resistance of bacteria to QACs, the study by Sundheim *et al* (1998) recommended that the use of higher temperature should be considered as an alternative or a supplement to using higher concentrations of QAC based disinfectants.

3.4.3 Peroxygen compounds

Hydrogen peroxide encompasses a broad-spectrum of disinfectants including hydrogen peroxide and peracid compounds (McDonell *et al*. 1999). Peracetic acid (PAA) or peroxyacetic acid, the most widely used of the peracid compounds, is a more potent biocide than hydrogen peroxide (McDonell *et al.* 1999). PAA has been known for its germicidal properties for a long time. However, it has only found food-industry application in recent years and is being promoted as a potential chlorine replacement (McDonell *et al.* 1999). PAA is commercially available in the form of a quaternary equilibrium mixture containing acid acetic, hydrogen peroxide, peroxyacetic acid, and water. PAA is relatively stable at strengths of 100 to 200ppm (Schmidt 2003). The desirable attributes of peracetic acid for disinfection are the ease of implementing treatment (without the need for expensive capital investment), broad spectrum of activity even in the presence of heterogenous organic matter, absence of persistent toxic or mutagenic residuals or by-products, no quenching requirement, small dependence on pH, short contact time, and effectiveness for primary and secondary effluents (Klaas *et al*. 2002, Kitis 2004). Furthermore, PAA has been found to be effective against biofilm bacteria especially if the biofilm contains food residues (Chmielewski and Frank 2003). The main disadvantages associated with peracetic acid disinfection are the increases of organic content in the effluent due to acetic acid and its pungent odour. Another drawback of the use of peracetic acid is its high cost, which is partly due to limited production capacity worldwide (Kitis 2004).

3.5 Factors affecting disinfection efficiency

The effectiveness of disinfectants is limited and much dependent on application conditions (Bessems 1998). The factors which control the efficiency of disinfectants are microbial type and growth condition; interfering substances; acidity-pH; temperature; contact time; and concentration (Bessems 1998, Chmielewski and Frank 2003).

3.5.1 Microbial type and growth conditions

Antimicrobial activity of a disinfectant varies greatly between different types of microorganisms and might also differ between different strains of the same species (Maillard 2002). Studies found that vegetative cells are more susceptible to disinfectant than spores (Kitis 2004) and adhered cells are less sensitive than plankton cells (Johnston and Jones 1995, Bredholt *et al.* 1999, Lindsay and von Holy 1999). Among vegetative bacteria, mycobacteria are probably the most resistant to disinfectant, followed by Gram-negative bacteria and Gram-positive bacteria, which is the most sensitive (Maillard 2002). The significant differences in the composition and structure of the cell and outer walls of these organisms can account for these phenomena (Maillard 2002).

3.5.2 Interfering substances

The efficiency of disinfectants is reduced in the presence of organic and inorganic matter. The influence of the protein load on the killing spectrum of different disinfectants was evidently proved in a series of studies such as Bessems (1998) and Lambert and Johnston (2001). Some disinfectants may also be affected by inorganic materials such as hard water salts. Among commonly used disinfectants in the food industry the disinfectant based on peracetic acid is relatively stable in use (National Seafood HACCP Alliance 2000). Although quaternary ammonium compound disinfectant is affected by water hardness it is less affected by organic matter (National Seafood HACCP Alliance 2000). In contrast disinfectant based on chlorine compounds is significantly reduced effectiveness by organic soils but is less affected by hard water (National Seafood HACCP Alliance 2000). Chemical reaction and spatial non-reaction are two main reasons that result in the reduction of disinfection efficiency (Lambert and Johnston 2001). In the former way, organic and inorganic material may compete with bacteria to react with disinfectants and thus the concentration of bactericidal compounds in aliquots is lowered (Lambert and Johnston 2001). Whilst, in the latter way, organic and inorganic material may form a spatial barrier such that microorganisms are protected from the effects of disinfectants (Lambert and Johnston 2001).

3.5.3 Acidity - pH

The acidity or pH of the make-up water is one of the factors significantly affecting the activity of some disinfectants (Schmidt 2003, Kitis 2004). Therefore to achieve the highest killing activity disinfectants should only be used within the pH range specified by the manufacturer (Springthorpe 2000).

3.5.4 Contact time, temperature and concentration

To be effective, disinfectants must find, bind to and transverse microbial cell envelopes before they reach their target site and begin to undertake the reactions, which will subsequently lead to the destruction of the microorganism (Kleperer 1982). Sufficient contact time is therefore critical to ensure disinfection and most general purpose disinfectants are formulated to reduce bacterial populations by at least 5 log orders within

5 minutes in suspension or a 3 log unit reduction in population of surface-adherent cells (Holah 1995). Contact time can be increased by applying the disinfectant as a foam or gel (Schmidt 2003). The relationship between time and efficiency is dependant upon the type of microorganism (Bessems 1998). There is a close relationship between contact time, temperature and bactericidal efficiency of some disinfectant (Schmidt 2003). The study by Taylor *et al*. (1999) found that at 20°C then 13 of 18 disinfectants tested were effective on *P. aeruginosa* whilst only 11 of them proved their effect at 10°C. The results by Tuncan (1993) also demonstrated that the efficiency of quaternary compound at 50ppm and lower concentration against *Listeria* sp. decreased considerably as the exposure temperature decreased. However, its effect was improved via increasing the contact time at cold temperature. Therefore, increasing temperature is also an alternative method that could be applied to improve the effectiveness of disinfectants (Langsrud 2003b). The range of temperature applied is typically from 5°C to 55°C. However in the majority of operations, disinfectants should offer a recognised performance level at ambient temperature (Schmidt 2003).

Concentration of disinfectant is one of the major factors in biocidal activity (Russell and McDonnell 2000). The relationship between microbial death and disinfectant concentration is not linear, but usually follows a typical biological sigmoidal death curve (Bessems 1998). The results by Tuncan (1993) indicated that the effectiveness of quaternary ammonium compound and chlorine on *Listeria* sp. was improved when the concentration was increased from 50ppm to 100-200ppm. Bessems (1998) found that at a constant test concentration, the rate of killing was increased with an increase in time and the relation between time and concentration for membrane-active disinfectant, i.e. quaternary ammonium compound was regulated by Gram-negative bacteria, whereas Gram-positive bacteria regulated the application of disinfectants having oxidising-properties, i.e. halogen containing disinfectant.

The recommendation concentrations of chlorine compounds, QACs and PAA commonly used in seafood plants are presented in Table 1.

Table 1: The recommended concentrations of common disinfectants (Huss 2003).

Sanitizers	Food contact surfaces	Non-food contact surfaces
Chlorine (ppm)	100-200*	400
Quats (ppm)	200*	400-800
Peroxyacetic acid (ppm)	200-315*	200-315

* The higher end of the listed range indicates the maximum concentration permitted without a required rinse (surfaces must drain)

3.6 Methods for testing disinfectant efficiency

3.6.1 Catalogues of test methods

There is a range of test methods for evaluating disinfectant efficiency. Although these methods differ in experimental detail they are all based on the same principle, which involves adding the test organism to a sample of disinfectant. The test mixture is sampled at the prescribed contact time and, following neutralisation of the disinfectant, the

number of survivors in the sample is estimated (Bloomfield *et al*. 1995). Disinfection tests are subdivided into suspension tests, carrier tests, surface disinfection tests and other practice-mimicking tests.

Carrier tests:

Carrier tests are the oldest tests. The process is as follows: the carrier (a silk or catgut thread, a penicylinder or a little stick) is contaminated by submersion in a liquid culture of the test organism; after drying it is brought into the use dilution of the disinfectant for a given contact time, after which it is cultured in a nutrient broth; no growth indicates activity of the product tested; growth indicates a failing. By multiplying the number of test concentrations and the contact times an overview of potentially active concentration-time relationships of the disinfectant is obtained. Example of a carrier test is the dilution test of the American Association of Official Analytical Chemists. The great shortcoming of the carrier tests is that the survival of the inoculums on the carrier is not constant and hard to standardise (Reybrouck 1998).

Suspension test:

The simplest disinfectant tests are the suspension tests. There are different kinds of suspension tests available: quantitative suspension tests and capacity tests.

A quantitative suspension test is the basic test in all contemporary testing schemes. This method involves adding a test organism to the dilutions of the relevant disinfectant. There is an intense contact between the disinfectant solution and organism cells. After several times of exposure, aliquots of solution are removed and the disinfectant is neutralised with a suitable quenching agent. Killing activity of test disinfectant is determined by comparing the numbers of organisms recorded after treatment to the numbers on untreated (control) suspension.

Although these tests are generally well standardised, i.e. the suspension tests of the European suspension test (Council of Europe 1987) and the proposal of the new basic bactericidal test of the European Committee of Standardisation (Holah 1995) they are less practice (Reybrouck 1998). The outcomes of these tests are not extremely accurate and they do not reflect the application conditions, especially because they do not take into account surface adhered bacteria (Council of Europe 1987; Holah 1995). Therefore capacity tests and practical tests were developed in order to simulate real-life situations, and to obtain results that offer more precise information on the effective use dilution for a given fields of application.

Capacity test:

The procedure of a capacity test can be understood as follows: each time a soiled instrument is placed into a container with disinfectant, or a mop is soaked in a bucket containing a disinfectant solution, a certain quantity of dirt and bacteria is transferred to the solution. The ability to retain activity in the presence of an increasing load is the

capacity of the disinfectant. Capacity tests simulate the practical situations of housekeeping and instrument disinfection. The best known capacity test is the Kelsey-Sykes test (Holah 1995).

Practical tests:

The practical tests under real-life conditions are performed after measuring the time-concentration relationship of the disinfectant in a suspension test. The best known practical tests are the surface disinfection tests (van Klingeren *et al.* 1998). The test schedule is as follows: the test surface (a small tile, a microscopic slide, a piece of PVC, a stainless steel disc, etc.) is contaminated with a standardised inoculums of the test bacteria and dried: then a define volume of the disinfectant solution is distributed over the carrier; after the given contact time the number of survivors is determined by impression on a contact plate or by a rinsing technique, in which the carrier is rinsed in diluents, and the number of bacteria is determined in the rinsing fluid. In order to determine the spontaneous dying rate of the organisms caused by drying on the carrier, a control series is included in which the disinfectant is substituted by distilled water; from the comparison of the survivor in this control series with the test series, the reduction is determined quantitatively.

There is an essential difference between a carrier test and a practical test: in the former case the carrier is submerged in the disinfectant solution during the whole contact time, whereas in the later case the disinfectant is applied on the surface for the application time and thereafter the surface is left to dry during the exposure (Reybrouck 1998).

3.6.2 Testing procedure

According new EU legislative requirements, such as Regulation EC No 648/2004 and Directive 98/8/EC, regarding disinfection products, all disinfectants have to pass a stringent testing procedure before they are accepted in the food industry (Easter 2003). The basic principles now widely accepted that the antimicrobial efficiency of a disinfectant is examined at three stages of testing (Bloomfield *et al*. 1995, Reybrouck 1998). The first stage is carried out in a laboratory in which disinfectant is verified whether a chemical compound or a preparation possessed antimicrobial activity. For these preliminary screening tests, suspension tests are considered. In the second stage of tests, the disinfection procedure is examined. It is determined under which conditions and at which use-dilution for a given application. The test used for this stage is often a practice test simulating real-life situations. The last stage is placed in the field with full sanitation procedure. The required test temperature for all disinfectant tests is 20°C as this represents a general, ambient temperature at which the majority of the disinfectant products would be expected to work (Holah *et al*. 1998).

3.6.3 The factors affecting the results of testing methods

The greatest problems in disinfectant testing are that of the repeatability (intra-laboratory spread) and of the reproducibility (inter-laboratory spread) (Bloomfield *et al*. 1995, Reybrouck 1998) since there are a lot of factors affecting the results of the tests.

Test organisms are always one of the significant parameters. The studies by Langsrud *et al.* (2003a, b) showed that the susceptibility of bacteria to disinfectant differs significantly depending on strains, growth rates, nutrient status and ambient environmental conditions. Therefore, the test bacteria chosen will greatly affect to the outcome of the disinfection test. In disinfectant testing pseudomonads and *Staphylococcal* species, usually *P.aeruginosa* and *S.aureus*, are often used because they have been shown to be the most frequent Gram-negative and Gram-positive species in the food industry environment (Holah *et al*. 2002) and due to their intrinsically high resistance to disinfectants (Russell and Chopra 1996, Langsrud *et al*. 2003b).

The method used to detect bacteria is also a significant factor. The conventional method used is swabbing and cultivation. However, the accuracy of this method is limited due to the swabbing procedure prior to cultivation, which may not have been efficient enough to detach and recover the cells from the test surface (Bredholt *et al.* 1999) and bacteria injured seriously after disinfection might not be visible on the plate (Johnston and Jones 1995, Lambert *et al*. 1998). Recently, methods based on epifluorenscence microscopy (Wirtanen *et al*. 1996), Malthus conductance (Flint *et al.* 1997), and turbidity endpoint (Lambert *et al*. 1998, Gilbert *et al*. 2001) have been proved to be perspective methods for detecting bacteria in disinfection tests. Despite the difficulties in harmonising the testing procedures (Reybrouck 1991) many attempts from scientists have been standardising the testing methods for disinfection (Holah *et al.*1998).

4 METHODOLOGY

The trial consisted of disinfecting assays with one phosphate buffer assay as a control. The main goal was to evaluate the bactericidal efficiency of three commercial disinfectants using a modified surface test as described by Guðbjörnsdóttir *et al.* (2005). As a means of simulating the practical conditions in fishery processing plants, lean and fat fish, cod juice and herring juice were used as the growth media for adhering test bacteria on the test surface. All tests were conducted in conditions of no water hardness.

4.1 Materials and methods

Bacterial strains and suspension preparation:

Bacterial strains were obtained from the culture collection at the Icelandic Fisheries Laboratories (IFL). Mixed cultures of *Pseudomonas putida* (IFL-H-03-302-14), *Serratia liquefaciens* (IFL-H-03-308-10) and *Shewanella putrefaciens* (IFL-H-03-302-12) isolated

from shrimp and fish processing plants in previous study by Guðbjörnsdóttir *et al*. (2005) were used as test organisms.

Stock cultures were maintained in Tryptic Soy Broth (TSB, Difco) and 20% glycerol at -70°C. Prior to use the cultures were grown in TSB at 22°C for 24 hours and sub-cultured twice.

To determine the initial number of cells, ten-fold dilution was prepared and 0.1 ml of selected dilution for each suspension was spread on Tryptic Soy Agar (TSA, Difco) and incubated at 22°C for 72 hours.

Preparation of steel coupons:

Flat, stainless steel coupons (type 304, Ra=0.75µ, 7x2.5cm) were used as the test surface. Before the first use they were soaked in 1M NaOH overnight to etch the surface clean and after that coupons were immersed in acetone for 1 hour to remove grease and cleaned again by washing machine. Each clean coupon was placed vertically in a glass tube and autoclaved at 121°C for 15 minutes (Guðbjörnsdóttir *et al*. 2005). The cleaning and sterilising procedures were repeated before re-using the coupons.

Chemical analysis:

The pH of the disinfectant solutions as well as the pH, salt, fat and protein contents of the fish juices were measured. Acidity (pH) was determined using Radiometer PHM80 (Copenhagen) at room temperature.

Salt contents of fish juices were measured according to the Titrino method (AOAC 16th ed. 1995 no.971.18), protein using the Kjeldahl method (ISO 5983-1997) and fat content using the Soxhlet method (AOCS Official method BA 3-88 and application noteTecator no.AN 301.1997) at the Chemical Laboratory of IFL.

Preparation of imitating media:

Fish juices of cod (*Gadus morhua*) and Atlantic herring (*Clupea harengus*) were used to simulate the processing conditions in relation to lean and fat fish. They were prepared by mixing one part minced fish with two parts deionised water followed by boiling for 2-3 minutes and filtering twice. Thereafter the pH and salt content of the fish juices were adjusted to the concentrations of cod and herring flesh. The fish juice was autoclaved at 121°C for 15 minutes. Sterilised fish juices were prepared in the first day, preserved in a refrigerator at 4°C and used for whole experiments.

Sanitising solutions:

Two products of quaternary ammonium compound and peracetic acid containing sanitizers were chosen to make the comparison in term of bactericidal capability with a

commercial available disinfecting product based on Sodium hypochlorite (15% active chlorine).

Disinfectant based on QAC was tested at concentrations of 0.25, 0.5 and 1.0% (v/v).

Disinfectant based on PAA was tested at concentrations of 0.25, 0.5 and 1% (v/v).

Sodium hypochlorite-containing disinfectant was conducted at 50 and 200ppm of active chlorine. To prepare a specific free chlorine solution (ppm) using sodium hypochlorite (NaOCl), the following formula was used (Ritenour *et al*. 2002).

$$G = \frac{A * B}{10 * C} \quad \text{(Equation 1)}$$

In which:

G: volume of NaClO-based disinfectant added,
A: Desired ppm of free chlorine,
B: Total volume in tank,
C: % concentration of NaClO in disinfectant used.

The stocks of disinfectant were stored in sealed bottles and prepared 2 hours before disinfection procedures were carried out. Dilution to target aqueous-phase concentration was accomplished with deionised water.

Artificial contamination:

Microbial adhesion was tested as described by Guðbjörnsdóttir *et al*. (2005). Thirty ml of each sterile fish juice was transferred into sterile glass tubes. One ml of relevant bacteria suspension with the population of 10^6 CFU/ml was transferred into the fish juice. The test was carried out at 19-21°C with shaking at *ca* 50 – 70 rpm for 24 hours and 48 hours in case of evaluating the adhesion of test bacteria or for 48 hours to test the bactericidal capability of disinfectants. Rinsing the cells adhered coupons three times with sterile water to remove unattached cells was followed by drying for 30 minutes at room temperature in a laminar air flow cabinet.

Enumeration bacteria on test surfaces:

Bacteria on test surface were enumerated using the swabbing method (Downes and Ido 2001) as follows. The test surfaces were scraped with two swabs coated with hydrophobic cotton, which were dipped in Bacto D/E neutralising broth (Difco) prior to neutralising the effects of disinfectant residue. The swab heads were broken off into a plastic bottle. The cotton swabs in the bottle were blended with 5 ml of Butterfield's buffer and shaken for 15 seconds to release the cells into the buffer. A series of logarithmic (ten fold) dilutions were prepared for each sample, plated onto Tryptic Soy Agar plus 0.6% yeast extracts (TSA/YE) for total count and incubated at 22°C for 72 hours. On the later stages during these experiments Yeast Extract was added to TSA to improve the recovery of the bacteria.

Evaluation adhesion capability:

After relevant contact time (24 or 48 hours), three coupons were scraped by hydrophobic cotton swabs and cultivated on TSA/YE plates to count the number of attached cells on the test surface, so-called total attachment number (A_C). Continually, the other three coupons were transferred to sterile glass tubes individually and then phosphate-buffer (30 ml) was poured and the coupon immerged for 10 minutes. After exposure with buffer, the coupon was rinsed gently three times with sterile water and then air-dried.

Samples for microbiological analysis were taken from the entire surface by the swabbing method described above.

In the present study, the adhesion degree (C_C) was investigated upon the percentage of cell numbers remaining on the test surface after being treated with buffer for a definite contact time (R_C) and total initial adherent number (A_C), using equation 2.

$$C_C = \frac{R_C}{A_C}.100 \quad \text{(Equation 2)}$$

The assays were repeated in the same procedure for herring juice using equation 3.

$$C_H = \frac{R_H}{A_H}.100 \quad \text{(Equation 3)}$$

In which:

The letter $_C$ and $_H$ signify cod and herring juices, respectively,
C: adhesion capability,
R: Retention number on the surface after treatment with buffer,
A: total initial adherent number.

Sanitation procedures:

The stainless steel coupons with attached cells after 48 hours of contact were disinfected with commercial disinfectants by modified surface tests in the condition of no water hardness as follows. The coupons with adherent cells were immersed in 30 ml aqueous solutions containing the sanitizers at a range of defined concentrations: (1) 0.25; 0.5 and 1 % QAC (v/v), (2) 0.25; 0.5 and 1% PAA (v/v), (3) 50 and 200ppm of available chlorine or in buffer for controls. The durations of disinfection were 1 and 20 minutes, and disinfection was carried out at ambient temperature around 19-21°C. After each contact time, the stainless steel coupons were removed aseptically from the test suspension in the glass tubes and rinsed gently with sterile water to remove residual disinfectant. After that, treated surfaces were allowed to air dry in a laminar air flow cabinet for 30 minutes at room temperature, samples for microbiological analysis were taken from the entire surface by the swabbing method described above.

Evaluation of disinfection efficiency:

The reduction in viability microbial effect (ME) values is calculated by subtracting the log of the viable count after disinfection from the log of the initial count using equation 4

ME = Nc – Nd (Equation 4)

In which:

Nc = log cfu count after treatment with water
Nd = log cfu count after treatment with disinfectant

To pass the test, disinfectants must achieve a three log reduction in viable counts (Mosteller and Bishop 1993, Holah 1995).

4.2 Statistical analysis

In this study, all tests were done by triplicate.

Analysis of variance (ANOVA) is performed using Number Cruncher Statistical Software (NCSS, 329, North 1000 East, Kaysville, Utah 84037). Evaluation is based on a level of significance of $P<0.05$. When the ANOVA showed a difference, Duncan's Multiple-Comparison Test is carried out to assess further differences between the means.

5 RESULTS

5.1 The control parameters used in the present study

The values of pH, salt, fat and protein content of cod and herring juice used in this study are measured and presented in Table 2.

Table 2: The values of pH, salt, fat and protein content of cod juice and herring juice.

Juice	pH	Salt content (%)	Fat content (%)	Protein content (%)
Cod juice	6.83	0.05	<0.1	1.61
Herring juice	6.49	0.29	0.40	1.34

In this study cod and herring juices were used to simulate the processing conditions of cod and herring. Therefore, the pH values and salt content of cod and herring juices should have the same values as those of cod and herring flesh (Table 3).

Table 3: The values of pH, salt, fat and protein content of cod and herring flesh.

	pH	Salt content (%)	Fat content (%)	Protein content (%)
Cod	6.6*	0.2*	0.5**	18.1**
Herring	6.5****	0.2-0.3***	12.3**	19.3**

(*) from Guðbjörnsdóttir (2004); (**) from Ólafur Reykdal (2005); (***) from IFL; and (****) measured in this project.

The disinfection tests were conducted at different concentrations whose pH values were measured in inuse solution and are presented in Table 4.

Table 4: The final pH values of the aqueous solutions containing disinfectants at concentrations used.

Disinfectant	pH				
	0.25%	0.5%	1%	50ppm	200ppm
PAA	3.57	3.40	3.23	-	-
QAC	6.83	6.52	6.29	-	-
Hypochlorite	-	-	-	10.7	11.2

5.2 Comparing adhesion of tested bacteria on a stainless steel surface

The results of adhering capability of mixed culture of *P. putida*, *S. liquefaciens*, and *S. putrefaciens* on stainless steel after a contact of 24 hours and 48 hours in cod juice and herring juice are show in Figure 2. The influences of different organic soils were assessed to improve understandings of adhesion and retention of bacteria in a fish processing environment.

High numbers of bacteria adhered readily to stainless steel coupons after 24 hours of contact time with cod and herring juices, approximately log 4 of cfu/ sample. There are slight increases in total adherence numbers ($p>0.05$) observed between 24 hours and 48 hours contact time, from log 4 and log 4.26 to log 4.47 and log 5.05 for cod and herring juice, respectively. The results also indicated that the amount of adherent cells reduced strongly after slightly rinsing with buffer in the procedure as mentioned particularly in the case of 24 hour contact time, from log 4 and log 4.47 to log 1.03 and log 1.34 for cod and herring juice, respectively. However, the reduction of adherent cells after a 48 hour contact time is much lower, decreasing from log 4.47 to log 3.62 and from log 5.05 to log 4.1 for cod and herring juice, respectively.

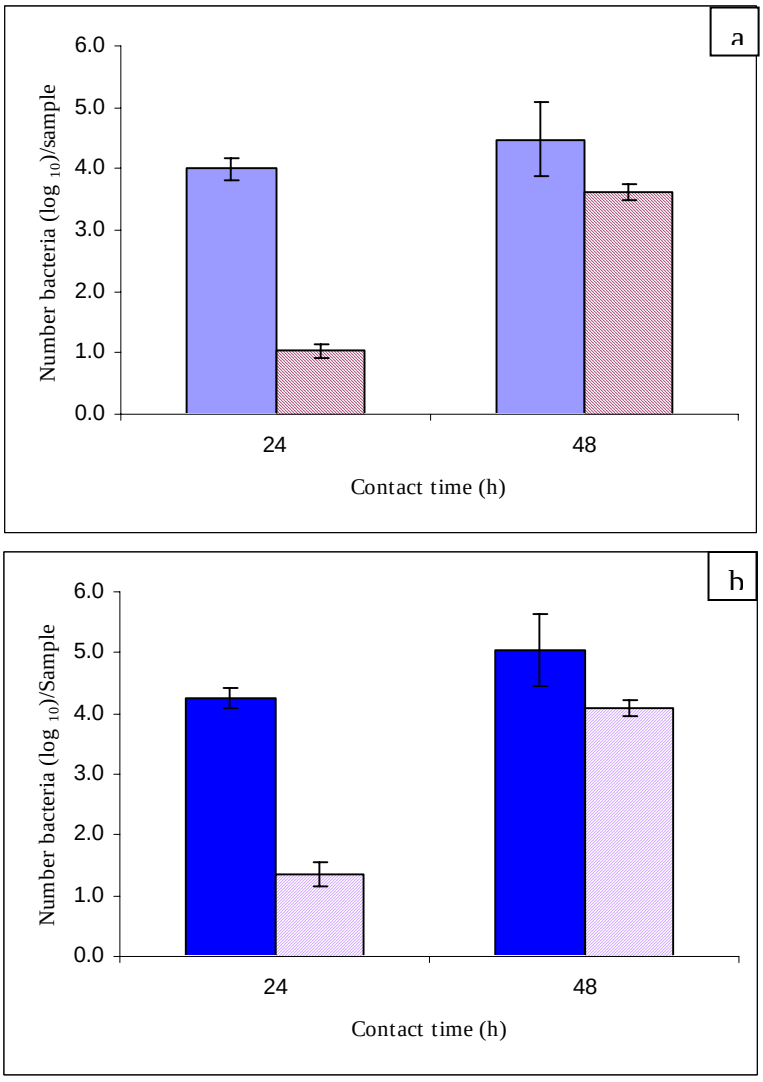

Figure 2: Bacterial number of mixed culture of *P. putida*, *S. liquefaciens*, and *S. putrefaciens* on stainless steel sample after rinsing with sterile water (open bar) and after extra 10 minutes treatment with buffer (hatched bar). Bars represent the standard error of the mean value of triplicate. a) cod juice, b) herring juice

There are significant differences ($p<0.05$) in the percentage of cells remaining on the surface after treatment with buffer following a contact time from 24 to 48 hours, increasing from 25.71% to 80.97% and from 31.54% to 81.15% for cod and herring juice, respectively (Table 5). Only slight differences were observed when comparing the cells remaining on the surface in relation to different fish juices, 25.71% and 31.54% after 24 hours or 80.8% and 81.15% after 48 hours for cod and herring juice, respectively.

Table 5: Percentage of mixed culture of *P. putida*, *S. liquefaciens*, and *S. putrefaciens* remaining on stainless steel surface after rinsing with buffer.

Fish Juice	The remaining cells (%)	
	Contact time (h)	
	24	48

Cod juice	25.71	80.97
Herring juice	31.54	81.15

5.3 Evaluating the bactericidal efficiency of three different disinfectants

Bacteria grown in media made of cod juice are more susceptible to the hypochlorite-based disinfectant as a few colony forming units were detected on the samples after a contact time of only 1 minute (Figure 3). On the other hand, the bacteria grown in media made of herring juice are rather resistant to this disinfectant. At either concentration used (50 and 200ppm of active chlorine) no considerable decrease in viable counts was observed after 1 minute contact time. Although the bactericidal effects of this disinfectant increase over time, the mean logarithmic number of residual bacteria is still rather high, log 2.26 and log1.73 for 50 and 200ppm after a contact time of 20 minutes, respectively.

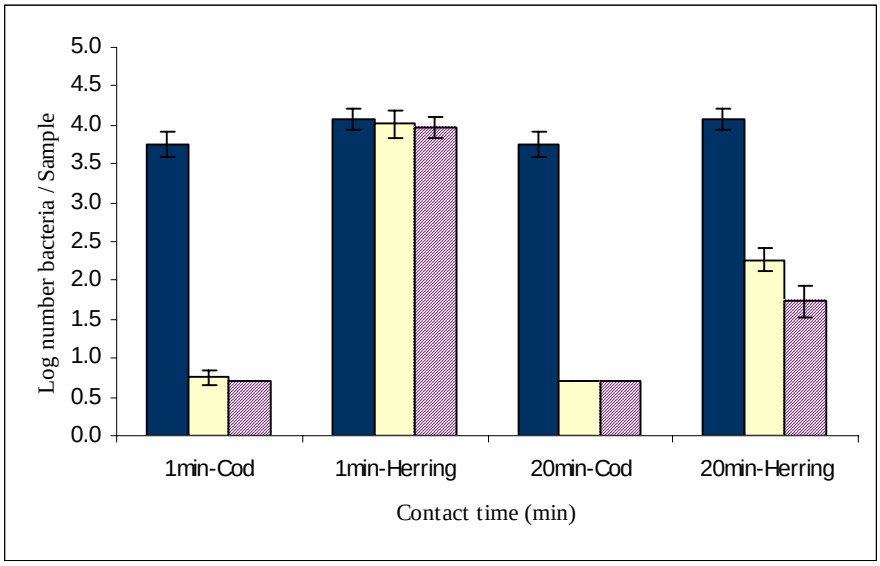

Figure 3: The bactericidal activities of disinfectant based on hypochlorite against mixed culture of *P. putida, S. liquefaciens*, and *S. putrefaciens* after contact times of 1 min and 20 min. Bars represent the standard error of the mean value of triplicate.

Control 50ppm 200ppm

The bactericidal activities of quaternary ammonium compound containing disinfectant are presented in Figure 4. Bactericidal efficiency is higher than that of the hypochlorite-based disinfectant especially on bacteria grown in herring juice ($p<0.05$). At concentrations of 0.5 and 1% (v/v) and a contact time of 1 minute very few colony forming units were detected in both cod juice and herring juice. However, at the lowest concentration (0.25%) it seem that this disinfectant is less effective on bacteria growth in herring juice compared to those in cod juice, mean logarithmic cfu/sample are log 3.59 and log1.18 over 20 minutes, respectively.

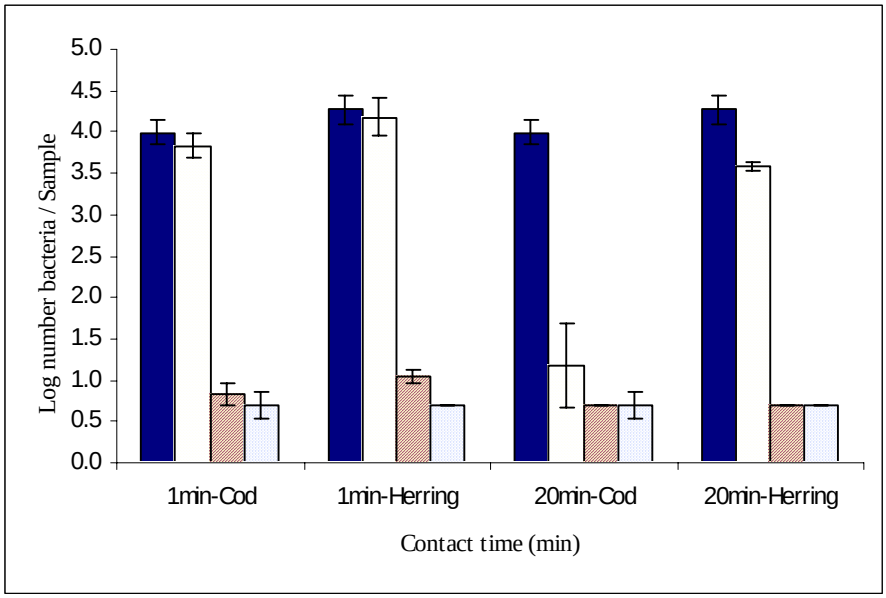

Figure 4: The bactericidal activities of disinfectant based on quaternary ammonium compound (QAC) against mixed culture of *P. putida*, *S. liquefaciens*, and *S. putrefaciens* after contact times of 1 min and 20 min. Bars represent the standard error of the mean value of triplicate.

Control 0.25% 0.5% 1%

Peracetic acid containing disinfectant showed good bactericidal activities on the mixed culture of tested bacteria (Figure 5). There are a lot of residue cells left on the surface after treatment with the disinfectant at 0.25% over 1 minute, however, at a concentration of 0.25% over 20 minutes as well as at a concentration 0.5 and 1% (v/v) 1 and 20 minutes of contact were found to eliminate virtually all bacteria in both cod juice and herring juice.

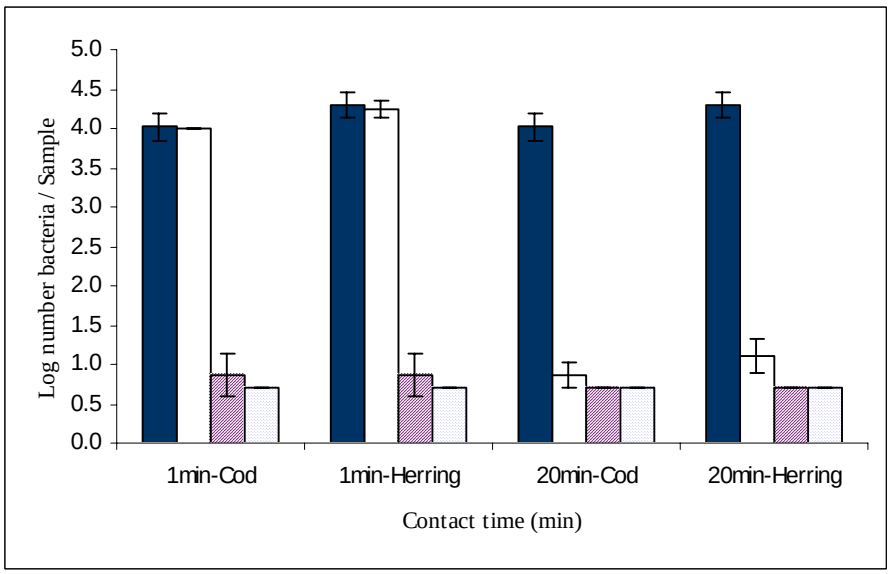

Figure 5: The bactericidal activities of disinfectant based on Peracetic acetic (PAA) against mixed culture of *P. putida*, *S. liquefaciens*, and *S. putrefaciens* after contact times of 1 min and 20 min. Bars represent the standard error of the mean value of triplicate.
Control 0.25% 0.5% 1%

From Figure 6-Figure 8 it can be seen that PAA-based disinfectant is the most effective disinfectant, followed by quaternary ammonium compound containing disinfectants and hypochlorite-based disinfectant. The last one was only effective against bacteria grown in cod juice. It is clear that although the three tested disinfectants are effective on bacteria grown in cod juice their efficiency is lower in herring juice. At the same application conditions regarding contact time media, concentration and contact time mean logarithmic reduction in viable cells after contact time to PAA is always equal or higher than those in case treated with QAC or hypochlorite. Particularly, at a concentration of 0.25% (v/v) over 20 minutes contact time for bacteria grown in herring juice, mean logarithmic reductions have significant differences between PAA and QAC, 3.14 and 0.65 log cfu/sample, respectively.

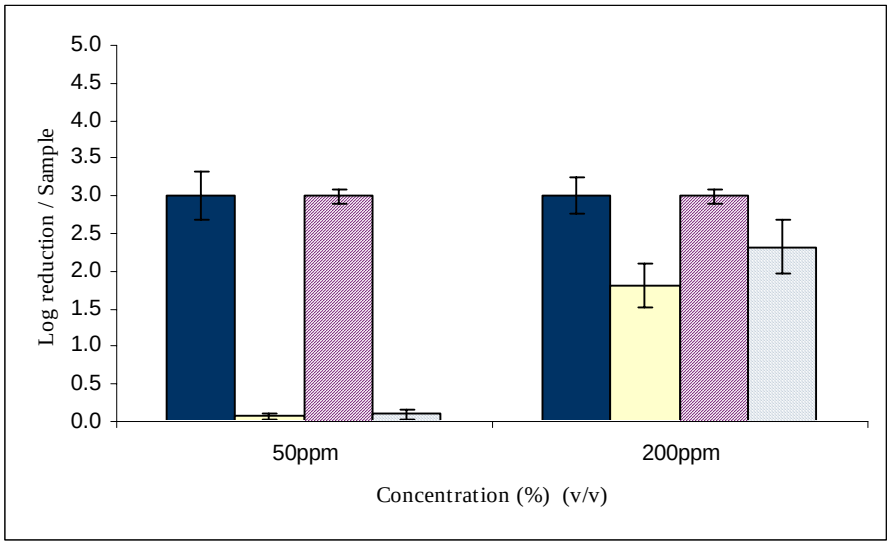

Figure 6: Mean logarithmic reduction in adherent cells of mixture of *P. putida*, *S. liquefaciens*, and *S. putrefaciens* after disinfecting with hypochlorite (HP). Bars represent the standard error of the mean value of triplicate.
1 min -cod 1 min - herring 20 min -cod 20 min - herring

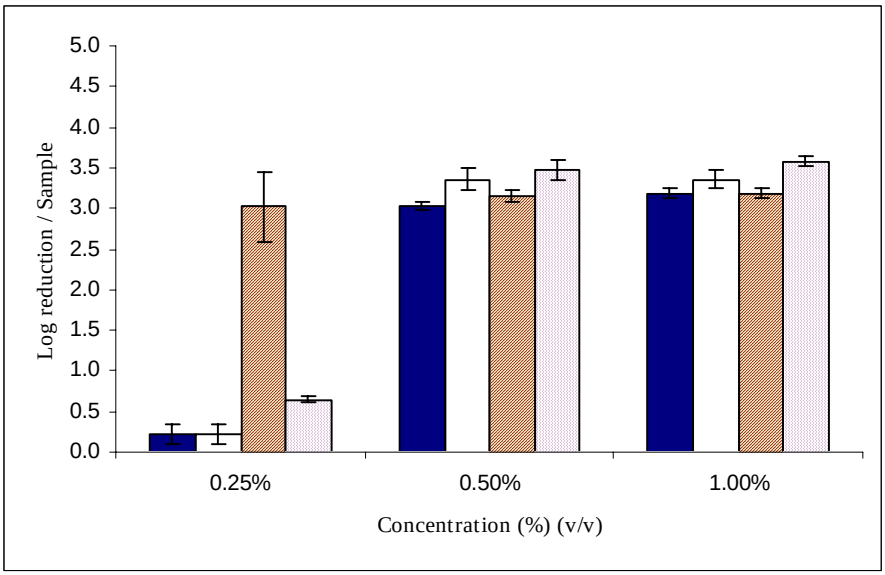

Figure 7: Mean logarithmic reduction in adherent cells of mixture of *P. putida*, *S. liquefaciens*, and *S. putrefaciens* after disinfecting with quaternary ammonium compound (QAC). Bars represent the standard error of the mean value of triplicate.

■ 1 min -cod □ 1 min - herring ▨ 20 min - cod ⁘ 20 min - herring

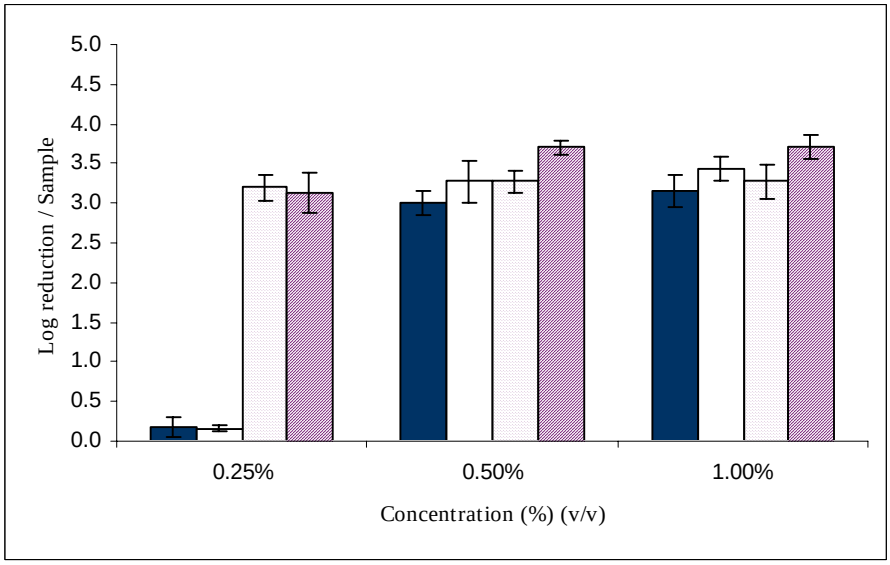

Figure 8: Mean logarithmic reduction in adherent cells of mixture of *P. putida*, *S. liquefaciens*, and *S. putrefaciens* after disinfecting with Peracetic acetic (PAA). Bars represent the standard error of the mean value of triplicate.

■ 1 min - cod □ 1 min - herring ⁘ 20 min - cod ▨ 20 min - herring

As seen in Table 6, in the presence of cod juice, most of test results pass with an exception of the cases of PAA and QAC solutions at concentrations of 0.25%% (v/v)

over 1 minute. Whilst, in the case of herring juice, only the solutions of PAA and QAC having concentrations equal or greater than 0.5% pass and hypochlorite containing disinfectant fails even at a concentration up to 200ppm of active chlorine over 20 minutes contact time.

Table 6: Disinfection results of the tested disinfectants containing PAA, QAC and hypochlorite against *P. putida*, *S. liquefaciens*, and *S. putrefaciens* after contact times of 1 and 20 minutes at different concentrations in the process conditions simulated by cod and herring juices.

Disinfectant	Fish juice	Concentration	Result	
			1min	20min
Hypochlorite	Cod	50ppm	P	P
		200ppm	P	P
	Herring	50ppm	F	F
		200ppm	F	F
PAA	Cod	0.25%	F	P
		0.5%	P	P
		1.0%	P	P
	Herring	0.25%	F	P
		0.5%	P	P
		1.0%	P	P
QAC	Cod	0.25%	F	P
		0.5%	P	P
		1.0%	P	P
	Herring	0.25%	F	F
		0.5%	P	P
		1.0%	P	P

P (Pass), 3-log reduction or greater in viable counts; F (Fail), less than 3-log reduction in viable counts; PAA, Peracetic acid containing disinfectant; QAC, Quaternary ammonium compound containing disinfectant.

6 DISCUSSIONS

6.1 The adhesion of mixed culture of bacteria on a stainless steel surface

The adhesion of mixed culture of three bacteria (*P. putida*, *S. liquefaciens*, and *S. putrefaciens*) were studied with different contact times, including cod juice and herring juice that were used to simulate fish processing conditions with respect to lean fish and fat fish.

In this study, it was expected that the level of adherent cells would increase over time of contact. However, in fact, the number of adherent cells on the sample in both cod and herring juices was not significantly different after contact times of 24 hours and 48 hours ($p>0.05$). Hood and Zottola (1997) studied the adhesion of *Salmonella typhimurium, L. monocytogenes, Escherichia coli* O157:H7, *Pseudomonas fragi and P. fluorescens* and noted that in some cases there was a linear increase in the log number of adherent cells with time. In other cases, the number of adherent cells remained constant over time. Hood and Zottola (1997) also showed that the medium, which produced the highest observed level of adherent cells, was different for each microorganism. It is not clear why the number of adherent cells does not increase over time. It is possible that the surface reaches such a saturation level that greater numbers of planktonic cells do not result in a greater number of adherent cells (Hood and Zottola 1997).

Although the initial number of adherent cells after a contact time of 24 hours and 48 hours were only slight different, the numbers were significantly different for bacteria retained after treating the samples with buffer ($p<0.05$). It has to be assumed that the washing method removed only non-adhering cells however Figure 2 showed that one simple wash actually does remove adherent cells. These findings indicate that the bond between tested bacteria and the stainless steel surface may be weak enough to be subject to “easy wiping off” of the surface. This phenomenon was also recognised by Norwood and Gilmour (1999). Many studies (Helke *et al.* 1993, Barnes *et al.* 1999, Bos *et al.* 2000, Parkar *et al.* 2001) have showed that the adherence and retention of microorganism to the surface is affected by some factors such as the bacteria motility or the transportation of the planktonic cells by gravity, diffusion or fluid dynamic forces from the surrounding fluid and the layer of protein covering the surface. Among these factors, the layer of protein covering the surface, so-called conditioning film has a major impact (Barnes *et al.* 1999, Parkar *et al.* 2001). The effect of conditioning film on bacterial adhesion may be either passive or active, depending on the type of bacteria, properties of surface and even on the type of protein. Bagge *et al.* (2001) found that, for *S. putrefacience* adhesion to stainless steel was facilitated by an initial organic layer, whereas the study by Hood and Zottola (1997) demonstrated that higher numbers of both *P. fragi and L. monocytogenes* bacteria were found on non-conditioned surfaces. Some food components, e.g., milk proteins, may actually decrease adhesion of bacteria (Barnes *et al.* 1999). Therefore, in relation to the results of this project, it might be suggested that there were certain substances in chemical composition of cod juice and herring juice (Appendix 1) that inhibited the firm adherence of tested bacteria to the sample surfaces. The inhibition of adhesion might be due to the presence of inhibitory macromolecules absorbed from

growth media components as stated by Barnes *et al.* 1999. This absorption resulted in the conditioning film. Conditioning film constitutes a weak link between a substratum surface and bacteria. Consequently, adhering microorganisms were more easily stimulated to detach when adhering to conditioning film than when adhering directly to the substratum. There may be another reason for the results observed in the present experiment. The simultaneous adhesion of mixed culture of three different bacteria might result in the weak adhesion of bacteria to stainless steel. Bagge *et al.* (2001) showed that the presence of *P. fluorescens* reduced the number of adhering *S. putrefaciens* bacteria. Similarly, McEldowney and Fletcher (1987) found that the simultaneous adhesion of a range of bacteria including *Acinetobacter calcoaceticus, Staphylococcus* sp. and *Coryneform* isolated from a canning factory in some cases, decreases the adhesion of others, depending the species combination and properties of the surface used.

In both culture media studied, the number of retained cells after treatment with buffer increased with contact time. The percentage of remaining cells of the test bacteria on the sample after 48 hours contact time increased significantly in comparison with those after 24 hours contact time ($p<0.05$). The time dependence may reflect the period required to induce the synthesis and the secretion of adhesive polymers by adhering cells. The results of studies by Dufrêne *et al.* (1996) and Norwood and Gilmour (1999) supported this view. Dufrêne *et al.* (1996) by using X-ray power spectrometer identified that after sufficient contact time (more than 24 hours) adhering cells produced extra-cellular material as essential proteins, which helped in the anchorage of cells to the surface and to stabilise the colony from the fluctuations of the environment. Norwood and Gilmour (1999) noted that prolonged contact between adhering cells and the substratum leads to the in situ secretion of proteins, which ensures cells anchorage.

Our findings indicated that adhesion of vegetative cells on stainless steel surface is weak. Cleaning routinely contact surfaces with running water in fishery processing plants is very necessary as it will result in a reduction of concentration disinfectants which, in consequence, will result in a positive acceptance of disinfectants. Based on the results of bacterial adherence, the tests to evaluate bactericidal efficiency of disinfectants in this study were carried out with 48 hours for growing bacteria so that the number of adherent cells on the sample after treatment with buffer is always higher than $1x10^3$cfu/sample.

6.2 Evaluating bactericidal efficiency of the three tested disinfectants

In this work, the results indicate that the bacteria grown in herring juice are more resistant to the tested disinfectants than those in cod juice ($p<0.05$). Adherent cells from a mixture of *P. putida*, *S. liquefaciens,* and *S. putrefaciens* grown in herring juice were not inhibited by disinfectant containing hypochlorite at either concentration as well as disinfectant based on and QAC at a concentration of 0.25% (v/v) whilst no viable count was detected after treatment in cod juice. In particular, the concentration of hypochlorite applied for herring (200ppm) was 75% higher compared to the concentration applied for cod (50ppm) however the effectiveness was significantly lower (Figure 6). A reason why it is able to detect the survivors in case of herring might be due to the presence of fat. A layer of fat was always observed only on the surface of samples, which was put in herring juice after

rinsing with the procedure as described above. This might suggest that fat is the main factor affecting on the bactericidal efficiency of the disinfectants, as the presence of fat might give the cells physical protection. This is in accordance with other studies. Taylor *et al*. (1999) showed that the tested disinfectant based on sodium hypochlorite at a concentration of 500ppm did not achieve a pass result under dirty conditions against *P. aeruginosa and Escherichia coli* O157:H7. The results by Bessems (1998) also noted that the concentrations of quaternary ammonium compound against Gram-negative bacteria *P. aeruginosa* could be reduced by 37.5% if clean conditions were fulfilled. These findings indicate that the difference in processing conditions regarding raw materials processed has a significant influence on the efficiency of disinfectants in the fishery industry. In addition they also indicate that if the cleaning procedure is performed properly to remove fat, the effectiveness of disinfection by using hypochlorite will be improved significantly.

The results of this project also indicate that if applying high enough concentrations of disinfectants based on PAA and QAC then the contact time does not have any visible effect on their efficiency against bacteria grown in both cod and herring juices. As at concentrations of 0.5% or greater, mean logarithmic reduction in adherent cells was always above 3.0, which reached the criteria of effectiveness (Mosteller and Bishop 1993, Holah 1995). However, at the lower concentration (0.25% (v/v)) their efficiency is improved considerably via increased contact time. Tuncan (1993) arrived at the same conclusion when studying inactivation of *Listeria* sp. attached on stainless steel. He found that the bactericidal activities of quaternary ammonium at high concentrations of 100-200ppm were independent to temperature and contact time. In contrast the effectiveness of lower concentration (50ppm) increased with an increase in temperature or contact time.

Many studies have been carried out to compare the antibacterial effects of common disinfectants in different application condition (Andrade *et al*. 1998, Rossini and Gaylarde 2000, Bagge-Ravn *et al*. 2003a). The effects of peroxyacetic acid and sodium hypochlorite on general hygiene and on *L. monocytogenes* were assessed in a salmon smokehouse by Bagge-Ravn *et al*. (2003a). The results showed that fog disinfection with peroxyacetic acid was more effective than foam application with sodium hypochlorite, 29 to 78% and 14 to 42% of the sample contained less than 10cfu per sample site, respectively. However, Rossini and Gaylarde (2000) had the opposite results when studying the effects of sodium hypochlorite and peracetic acid on *Escherichia coli, P. fluorescens* and *S. aureus* isolated from chicken carcasses adhering to stainless steel. The tests were conducted at the concentration of 250 or 1000 mg l^{-1} for peracetic acetic and 100 or 200 mg l^{-1} for hypochlorite over 10 minutes. In all cases, sodium hypochlorite was more effective than peracetic acid in killing or removing the adherent cells. The results also showed that peracetic acid had good activity against *P. fluorescens* whereas it was less effective against *S. aureus*. At the concentration of 250 mg l^{-1} the reduction in viable adhered cell numbers was over 90% for *P. fluorescens* and only slightly over 50% for *S. aureus*. Whilst, PAA disinfected *S. aureus* $2x10^2$ times faster than hydrogen peroxide and for *P. aueruginosa* the value was $1.2x10^4$. Andrade *et al*. (1998) had the conclusion that peracetic acid and peracetic acid plus an organic acid were more effective against

Enterococcus faecium attached to stainless steel than sodium hypochlorite, quaternary ammonium, organic acid and anionic acid.

It is difficult to make direct comparisons between these results because there were great differences in methods used as well as application conditions. However, these findings may suggest that PAA, QAC and hypochlorite are effective disinfectants against most Gram-negative bacteria. However, during their application the user has to consider several advantages and disadvantages. Quaternary ammonium compounds (QACs) have been used widely as disinfectants in seafood processing environment in developed countries such as the UK (Holah *et al*. 2002), Norway (Bore and Langsrud 2005). Because of its low toxicity non-corrosiveness and high surface activity (Langsrud and Sundheim 1997). However, several reports have described intrinsic and acquired resistance to these compounds especially among some Gram- negative species (Langsrud and Sundheim 1997, Langsrud *et al*. 2003). Although hypochlorite problems with resistance to bacteria are not as big as QACs, it has other problems with toxic by-products and corrosiveness (Fiessinger 1985, Shang and Blatchley 2001) whilst the disadvantages of PAA are high costs and irritating odour. The selection of a disinfectant must be considered by combining effective antimicrobial activity with minimal toxicity and cost (Holah *et al*. 2002). To improve efficiency of disinfection routines and avoid build-up of resistance, disinfection rotation has been recommended (Holah *et al*. 2002). In Icelandic seafood processing plants the efficiency of sanitation procedures has been achieved via applying alternatively QAC and an oxidative disinfectant, usually hypochlorite. It is a useful experience for applying disinfectants in Vietnamese fishery industry.

7 CONCLUSIONS AND RECOMMENDATIONS

The use of hypochlorite as a disinfectant in the presence of fat (herring juice) in this study showed a weak bactericidal efficiency. PAA and QAC were more effective than hypochlorite both for cod and herring juice (mimicking the processing conditions of lean fish and fat fish, respectively). The bactericidal effects of these three disinfectants were improved with increased time or concentration. The results from this project indicate that the type of raw material produced must be considered as being a predominating parameter affecting the time-concentration relation of the applied disinfectants. It also confirmed that if cleaning steps are implemented properly to remove soil residues before disinfection the success of disinfection operations will improve and can be achieved with the application of a lower level of disinfectants.

From the results of this project it can be stated that the disinfection efficiency in fisheries processing plants in Vietnam can be improved via applying alternatives to chlorine containing disinfectants such as QACs or PAA and rotation disinfection should be considered to improve the efficiency of disinfection operations in fish processing plants as well as to save costs and to reduce the effects of disinfection by products to the environment.

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APPENDIX

Appendix 1: The muscle extractives of cod and herring (Shewan, 1974)

N°	Compound in mg/100wet weight[1]	Fish Cod	Fish herring
1.	Total extractives	1200	1200
2.	Total free amino acids:	75	300
	Arginine	<10	<10
	Glycine	20	20
	Glutamic acid	<10	<10
	Histidine	<1.0	86
	Proline	<1.0	<1.0
3.	Creatine	400	400
4.	Betaine	0	0
5.	Trimethylamine oxide	350	250
6.	Anserine	150	0
7.	Carnosine	0	0
8.	Urea	0	0