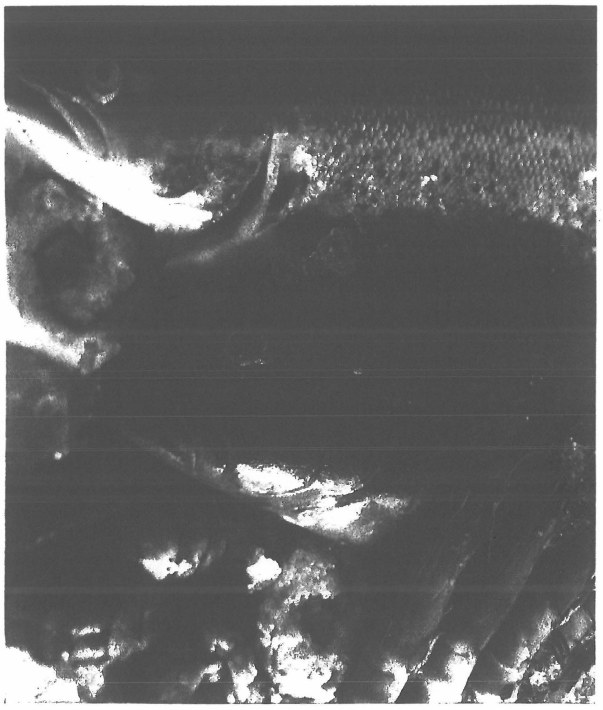
Final Report
to National Seafood Centre

NSC 97/482

# Effects of Stabilised Chlorine Dioxide on Reducing the Rate of Seafood Spoilage and Malodours



FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Quantum Control Pty. Ltd.

CENTRE FOR FOOD TECHNOLOGY

Project no. NSC 97/482

# Effects of stabilised chlorine dioxide on reducing rate of seafood spoilage and malodours.

## Project Applicant:

Lionel Freedman 07 5564 9333
Managing Director
Quantum Control Pty Ltd
PO Box 7143
GCMC Q4217

## Principal Investigator:

Sue Poole 07 3406 8689
Senior Seafood Microbiologist
Centre for Food Technology
Qld Department of Primary Industries
19 Hercules Street
Hamilton Q4007

## Objectives:

- To evaluate the efficacy of stabilised chlorine dioxide use in ice for reducing microbial loads on chilled seafoods.

- To determine the keeping quality benefit gained and malodour reduction by storing seafood product in ice which incorporates stabilised chlorine dioxide.

## Funds expenditure:

This project was supported by funding from the National Seafood Centre under the D-Program (maximum funds to $7 000). A full disclosure of funds expended is attached in Appendix1.

## PROJECT SUMMARY

Stabilised chlorine dioxide, marketed as AquaPlus in Australia, is an aqueous solution containing 5% available chlorine dioxide. As a strong oxidant, AquaPlus is stated to be a very efficient sanitiser and odour-neutralising substance (Appendix 2). Stabilised chlorine dioxide solution can be activated by lowering the pH which neutralises the buffering system of the solution and allows maximum efficacy of the oxidant action. AquaPlus carries EPA, USDA and FDA approvals for various fields of use.

This project sought to determine the effectiveness of incorporating chlorine dioxide into ice at a concentration of 20ppm AquaPlus for reducing microbial loads of chilled seafoods stored in the ice. The chlorine dioxide was used in the stabilised form with no prior activation of the compound. Increase in microbial population on both green tiger and cooked Bay prawns was delayed during storage in chlorine dioxide ice. This was not the case for the whole whiting, where bacterial growth appeared to occur at the same rate for fish stored in unmodified ice and chlorine dioxide ice.

The project constraints of this research allowed only one concentration of AquaPlus to be investigated. However, the findings of the work indicate that increasing the concentration of AquaPlus above 20ppm for incorporation into ice may provide greater benefit in reducing rate of bacterial growth. It is also worth considering the use of crushed ice or slurry to allow greater direct contact of chlorine dioxide with product surface and bacteria.

Ice incorporating AquaPlus was very effective in reducing odour of both green and cooked prawns. Odour intensity remained minimal for prawns stored in chlorine dioxide ice whereas there was a characteristic increase in intensity for prawns stored in unmodified ice. This finding indicates a definite benefit in storing prawns in the presence of chlorine dioxide ice.

The overall sensory rating of the green tiger prawns was consistently better than that for prawns stored in unmodified ice, although the difference was small. Additionally, blackspot on green prawns was reduced in the presence of chlorine dioxide ice. Cooked Bay prawns were rated with similar sensory quality loss irrespective of the presence or absence of chlorine dioxide.

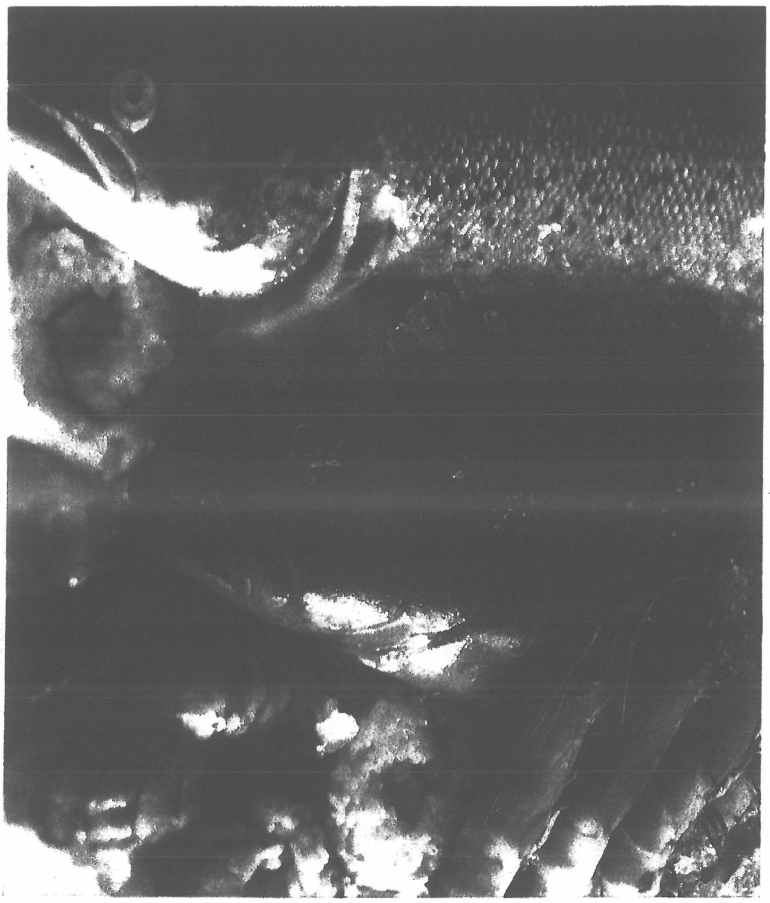
In contrast to the prawn results, whole whiting stored in chlorine dioxide ice did not demonstrate a reduction in odour intensity. Quality loss measured by sensory parameters was not impeded in the presence of chlorine dioxide either. It was beyond the constraints of this investigation to determine the reason for the findings with the whole whiting. It is possible that the unexpected results could have been decreed by the initial condition of the fish at purchase, as well as differences between fish used for the two treatments.

**APPENDIX 1.**

## Expenditure of funds

| | |
|---|---|
| Professional fees (47hr @ $90/hr) | $ 4 230 |
| Sample analysis (40 samples @ $15/sample) | $ 600 |
| Supply and operation of olfactometer | $ 1 382 |
| Supply of AquaPlus | $ 52 |
| Purchase of seafood | |
| green prawns | $ 236.90 |
| cooked prawns | $ 12.60 |
| whiting | $ 166.50 |
| **TOTAL** | **$ 6 680.00** |

# EFFECTS OF STABILISED CHLORINE DIOXIDE ON REDUCING RATE OF SEAFOOD SPOILAGE AND MALODOURS



**REPORT FOR**

**QUANTUM CONTROL Pty Ltd**

**Prepared by**

**SUE POOLE and ROSS NAIDOO**

**April 1998**

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## INTRODUCTION

All seafoods progressively deteriorate during chilled storage and micro-organisms are known to be the main cause of this spoilage. Bacterial growth produces enzymes which are capable of breaking down components of the seafood and the resulting by-products are indicative of spoilage (Shewan and Murray, 1977; Thatcher, 1973). Additionally, spoilage occurs from chemical and biochemical changes induced by enzymes inherent in seafoods (Love, 1980).

This degradative process occurs even when the temperature of the seafood product is reduced to near 0°C and hence when seafood is stored in ice.

All sectors of the seafood industry are increasingly aware of the importance providing excellent quality seafood product to maximise returns. Wholesalers and retailers are becoming particularly conscious of the demand by the consumer, who will pay a premium for the top quality product. One of the commonest negative judgements made by consumers is based on off-odours being present, both relating to the seafood product and also the retail premises.

Product spoilage remains a primary concern with respect to loss of revenue within the industry. In recent discussions with several large seafood retailers the loss, termed shrinkage, was mentioned to occur at a rate as high as 10% (pers. comm. 1998).

Chlorine dioxide, marketed in Australia as AquaPlus, is a stabilised form of the gas chlorine dioxide in solution. It acts as a strong oxidising agent and was found to be seven times more potent than aqueous chlorine in killing bacteria in chilling water used for poultry processing (Lillard, 1979). Chlorine dioxide maintains bactericidal activity far longer than does aqueous chlorine (Wei-Fang Lin *et al.*, 1996) and unlike chlorine, it does not chlorinate organic compounds to form potentially toxic reaction byproducts (Sen *et al.*, 1989; Wei *et al.*, 1987).

Chlorine dioxide has been widely used overseas in many applications: numerous sectors of the food industry, treatment of potable water, brewing and fermentation industries, for animal rearing and confinement, within health care facilities and for industrial use including treatment of waste water, biofilm removal and odour control. Chlorine dioxide (AquaPlus) carries EPA, USDA and FDA approvals for various uses. In Australia, in 1992, the Western Australian Health Department approved the use of stabilised chlorine dioxide as a disinfectant and sanitiser for food contact surfaces and for control of bacteria within the seafood and poultry processing industries, for ice making machines and ice, as well as for humidifier systems. Full Australia-wide approval has not been sought to date.

This investigation was undertaken to determine the efficacy of AquaPlus incorporated into ice for reducing the microbial loads on chilled ice-stored seafoods and thereby the potential for maintaining the quality of seafoods for a longer time.

## METHODS

### Preliminary experiment

The bactericidal activity of AquaPlus at various concentrations, 0 to 50,000ppm was determined against *Staphylococcus aureus* ATCC 25923, a reference strain used for antimicrobial assays. A lawn of the micro-organism was swabbed evenly onto the surface of nutrient agar (Oxoid No.2). Two random sectors of the plate were marked and 0.1ml of either $ClO_2$ solution or sterile water was delivered to the marked sector. Plates were initially read after 18h incubation at 30°C. The results of bacterial growth at this stage appeared unusual and anomalous, hence an additional repeat 0.1ml aliquot was delivered to the marked sectors and the plates incubated for a further 18h at 30°C. The final growth reading was taken at this time.

### Ice production

For incorporating AquaPlus into ice, a final concentration of 20 ppm was recommended by the supplier marketing the product, based on procedures used overseas. The concentration of the $ClO_2$ was taken as 7.5% as per supplier specifications. Eighty litres of town water (+/- 500 ml) was placed in a two hundred litre plastic container. Using a pipette 1.6 ml of the 7.5% AquaPlus stock solution was added to give a final solution of 20 ppm. The solution was agitated using clean plastic piping by stirring in clockwise and counter-clockwise direction for twenty revolutions.

Eight to ten litres of $ClO_2$ solution was transferred to clean stainless steel trays and then frozen at -25°C overnight. The solid sheet ice was reduced to standard flaked ice size by a Hall mince-a-mix (Lawerence Hall & Sons, Mortlake, Australia), the inner chamber of which had been pre-cooled for one hour. The $ClO_2$ ice was stored at -25°C until required.

### Seafood Treatments

A total of 140 whole whiting (gut in), 230 whole green tiger prawns and 230 whole cooked Bay prawns were purchased directly from a commercial wholesaler. These samples were then divided randomly into two equal quantities for storage in unmodified and $ClO_2$ ice. Rectangular plastic drain bins were used to store the seafood with product packed surrounded by ice. The seafood was packed so as to minimise the contact between individual samples whilst maximising product surface area contact with the ice. Packing, storage and sampling was carried out at 2°C. Storage was for 8d.

Samples of seafood and drip water were taken on a daily basis for microbiological analysis. Each ice treatment was checked daily and re-icing carried out as required.

### Microbiological analysis

*Ice sample preparation*

Three samples of ice were taken aseptically, held at room temperature for three hours to melt and appropriate 10-fold dilutions made in 0.1% peptone. In addition, two 100ml melted ice samples were filtered through a millipore filtration unit using a 22µm membrane. The membrane was transferred aseptically to separate petri plates containing nutrient agar.

*Seafood sample preparation*

Each sampling day three individual whiting, green tiger prawns and cooked bay prawns were aseptically removed from each ice treatment and submitted for mesophilic and psychrotrophic counts.

For the fish, surface swabs were taken using a $10cm^2$ sterile template and swabs were composited together as one sample. For both green and cooked prawns, a 10g subsample was taken of the three individual prawns and macerated for 60 seconds in a Seward BA6021 type stomacher with peptone diluent. All samples were stomached for 60 seconds in and serial dilutions made as appropriate.

*Microbiological enumeration:*

Approximately 15ml of sterile nutrient agar (BBL), 45° -50°C was mixed with 1ml aliquots of sample dilution in petri dishes and allowed to set. Incubation was at 30°C for 72h to enumerate mesophilic bacteria and at 4°C for 14 days to enumerate psychrotrophs. Plates were then counted and numbers recorded as colony forming units per gram(cfu/g).

**Odour Intensity**

Odour intensity of the stored products was determined using a Max Winders and Associates Butanol Olfactometer. This instrument is based on a design developed by researchers at Texas A&M University and uses a 1-butanol reference standard to measure odour levels.

A predetermined level of 1-butanol vapour, ranging from 1.25 ppm to 80 ppm, is delivered to the panellist by mask. The panellist first sniffs the test odour and then compares the intensity of that odour to the intensity of the butanol level delivered, rating it as higher or lower. Results are recorded as that intensity of butanol which is equivalent to the test odour intensity.

For this investigation, 5 panellists were used each sampling day of the trial and the results from all panellists were averaged for that day.

**Sensory Assessment**

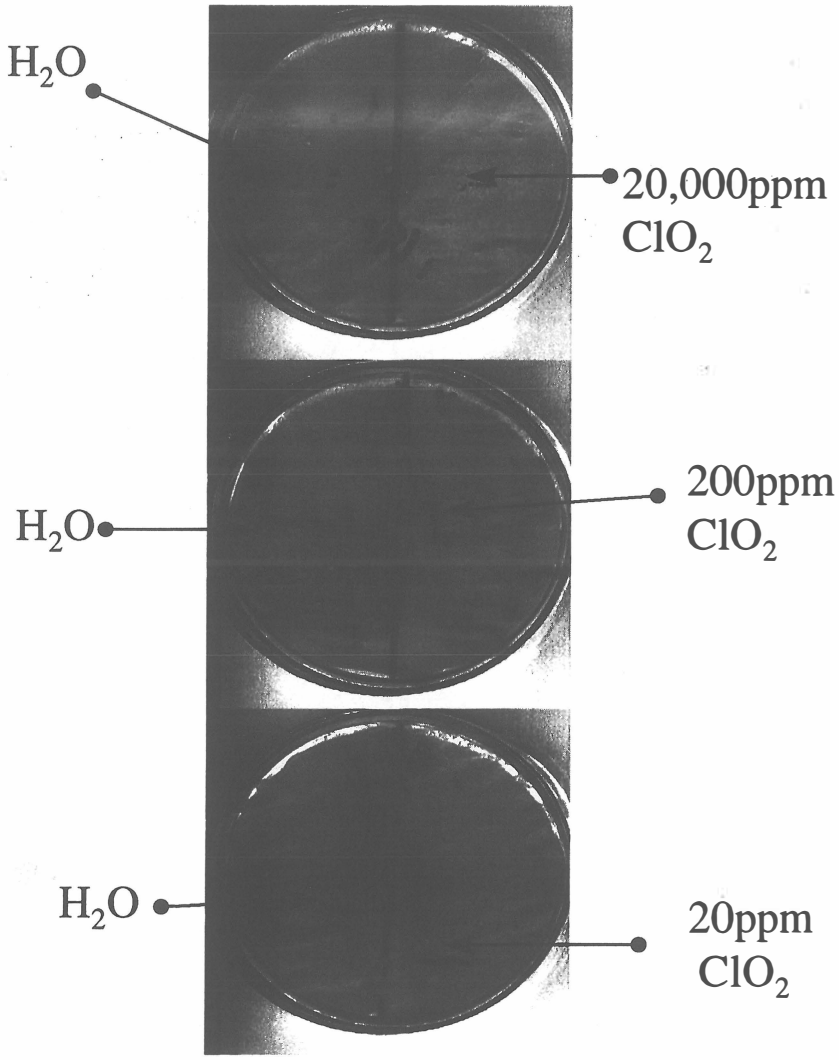
Three samples of fish and prawns were removed from each treatment and presented on ice for sensory evaluation by a standardised group of five experienced panellist. A standard survey assessment sheet was used to quantify the quality of both the fish and prawns. All the results were recorded as an overall average in demerit scores.

## RESULTS

### Preliminary experiment

A quick one-off check of the inhibitory effect of AquaPlus against bacteria was tested under a simplistic assay system used for determining antimicrobial activity. After incubation, the plate showed that the $ClO_2$ did not inhibit growth of *Staphylococcus aureus* at a concentration of 2,000ppm. This result was unexpected against the background information available from the company literature. However, the assay system used was not completely appropriate for testing microbial growth inhibition in this way as it was not suitable for solution and contact time was insufficient against a very high log phrase population growth. Despite these demands of the assay method, reapplication of solutions to the marked sectors demonstrated that growth was inhibited at $ClO_2$ concentrations as low as 20ppm (Figure 1). This confirmed the suitability of using AquaPlus at this concentration.

*Figure 1*

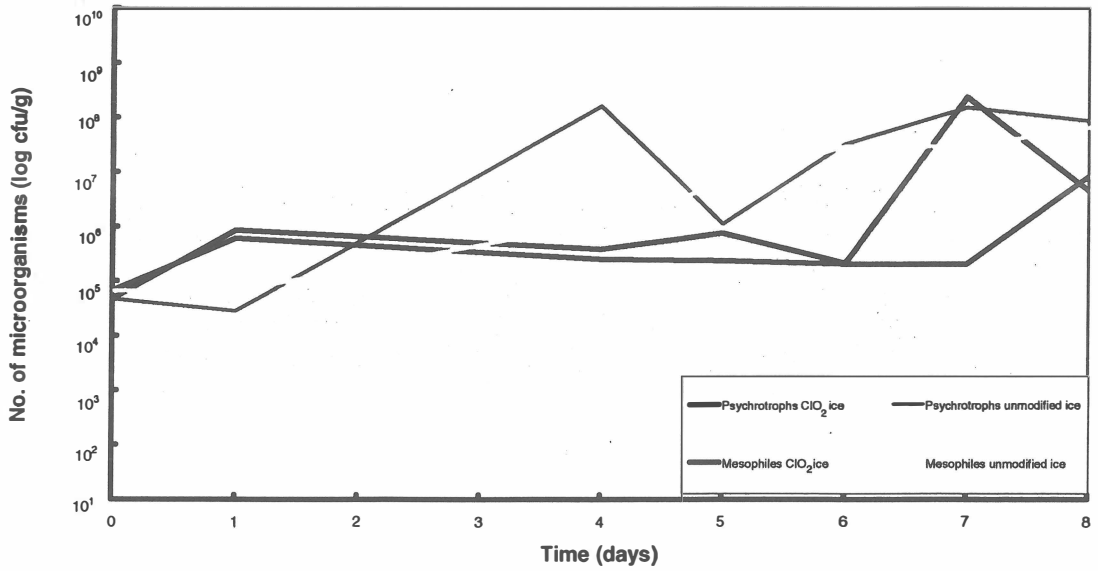


## Iced Storage Treatments

GREEN TIGER PRAWNS.

The microbial loads of green tiger prawns stored in ice are presented in Figure 2. Both psychrotrophs (bacteria able to grow at chill temperatures) and mesophillic bacteria (those species unable to grow at low temperatures) were enumerated separately. This was to establish the relative proportion of the microbial population able to grow at ice temperatures. These bacterial species commonly have more significance with respect to seafood spoilage during chill storage. The initial load carried on the prawns was less than $10^4$ cfu/g which is indicative of fresh well handled prawns. Figure 2 illustrates that both psychrotrophs and mesophiles were reduced in the presence of $ClO_2$ compared to counts obtained from prawns stored in unmodified ice.

*Figure 2* **Microbial load of green tiger prawns**




The psychrotroph counts determined fluctuated widely from day to day and this is possibly to be due to variation of bacterial load carried by individual prawns, dependent on catch and handling history. However, results indicate that green prawns stored in $ClO_2$ ice retain acceptably low microbial loads for at least a further 6 days from purchase whereas those stored in unmodified ice reached equivalent numbers at 4 days.

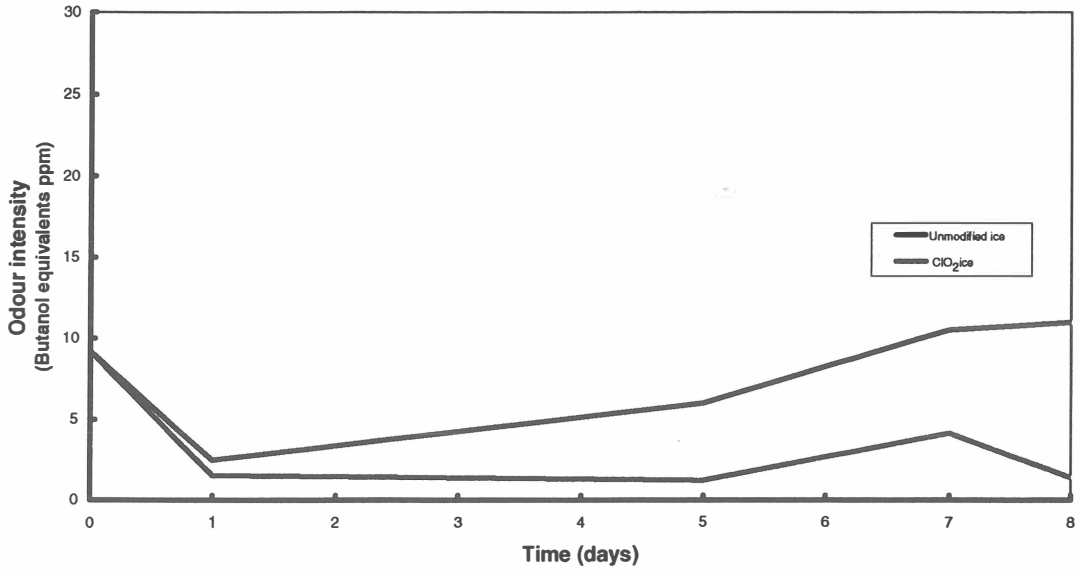
The overall sensory rating of green prawns confirmed the initial good quality (Figure 3), then deteriorated over time following a characteristic pattern. Prawns stored in $ClO_2$ ice were consistently rated as better than those stored in unmodified ice, but the difference was only slight.

The odour intensity of the stored prawns, as measured relative to butanol concentration, decreased initially which is characteristic of the spoilage pattern for prawns. For the prawns stored in unmodified ice there was a gradual increase in odour intensity (Figure 4) typical for prawns stored in ice over time and attributable to development of off-odours

produced from enzymatic action. In contrast, those prawns stored in the presence of $ClO_2$ remained odourless for most of the storage period.

*Figure 4*

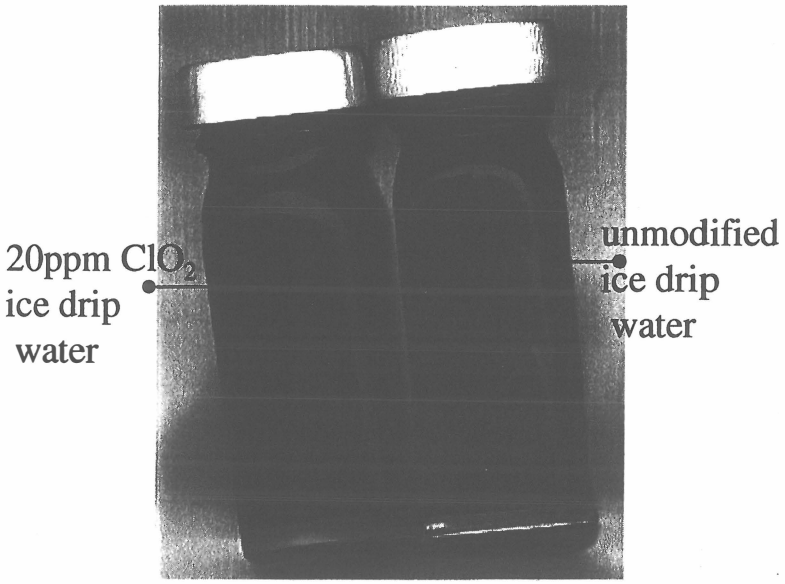
**Odour intensity for green tiger prawns**




As well as the parameters evaluated, additional observations were made about the degree of blackspot on the prawns. Throughout the storage period, the raters consistently judged the green prawns stored in $ClO_2$ ice to have less blackspot evident than those stored in unmodified ice. Interestingly, the dripwater from the $ClO_2$ iced prawns was significantly blacker than that from the unmodified ice (Figure 5 ). It is possible the strong oxidising nature of $ClO_2$ was enhancing the phenyloxidase reaction which naturally occurs with prawns.

*Figure 5*

Drip water from green tiger prawns




COOKED BAY PRAWNS

Although the purchased product was assumed to be fresh, initial microbial loads on the cooked mixed Bay prawns were high at $10^6$ cfu/g (Figure 6). This microbial load is considered to benchmark the limit of practical storage life of seafoods, with a population of $10^7$ cfu/g taken as the rule of thumb indicating spoilage.

Once stored in ice, there was no increase for the first day, however by day 4 psychrotrophic bacterial numbers had increased dramatically. Unusually, the mesophile population did not increase for the first 5 days of storage, but then population growth followed a similar growth pattern to the psychrotrophs.

*Figure* 6

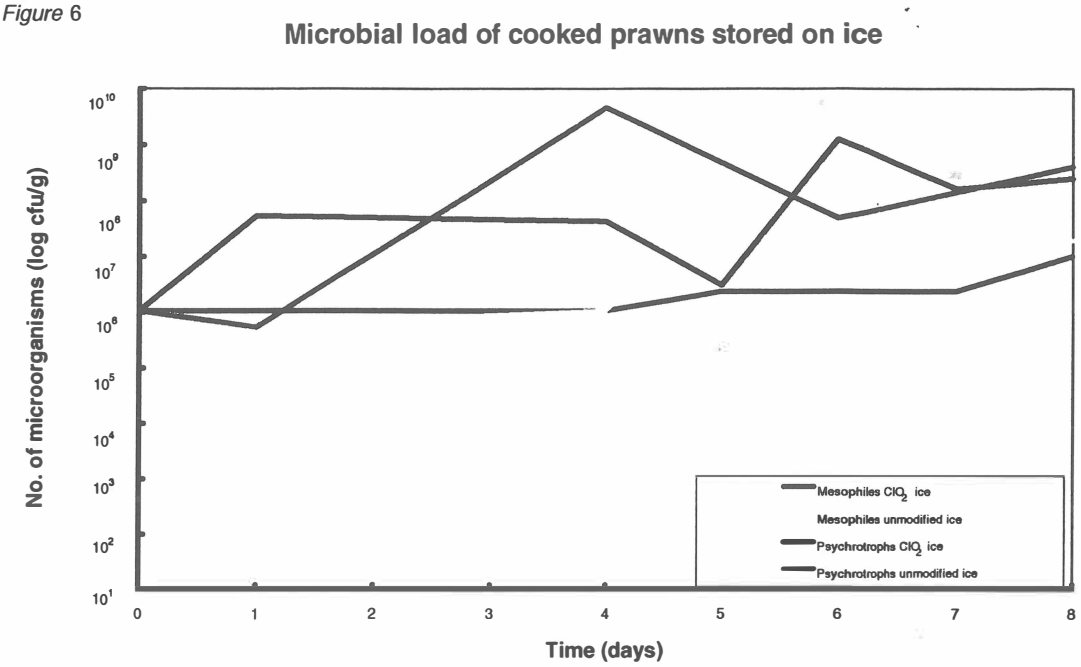
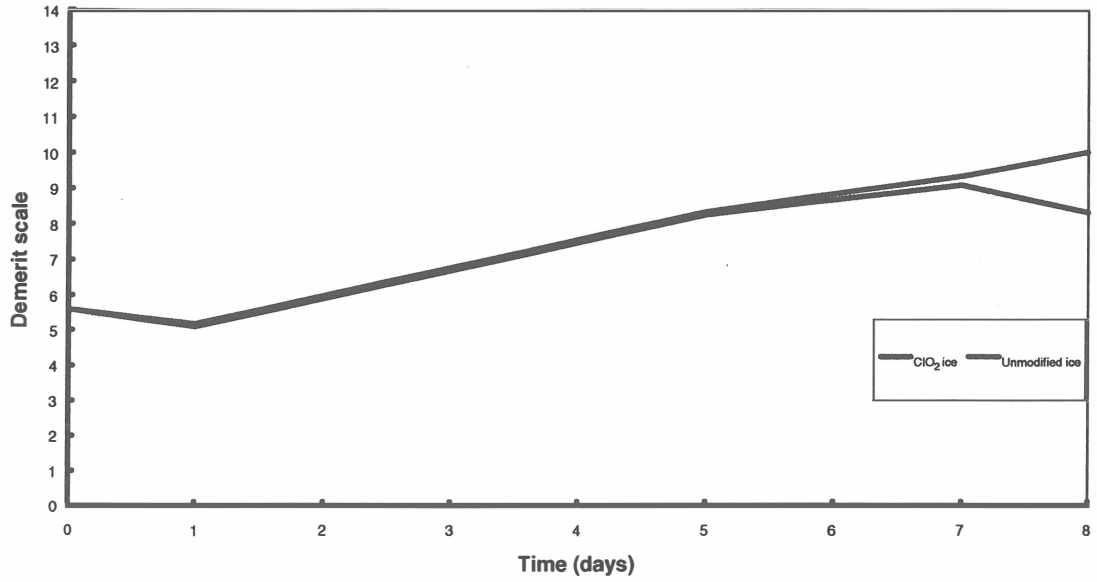



Figure 6 illustrates obvious differences in microbial loads between prawns stored in $ClO_2$ ice and unmodified ice for the entire storage period. Psychrotrophic bacterial numbers on prawns stored in the presence of $ClO_2$ are 2 log cycles lower after 4 days storage than prawns stored in unmodified ice. For mesophilic bacteria $ClO_2$ presence has a significant effect in holding the population at a level similar to the initial level present. This effect was maintained for 7 days storage and was in contrast d to the population growth pattern observed on prawns stored in unmodified ice.

Sensory ratings of the physical condition of the mixed Bay prawns (Figure 7) demonstrated steady deterioration in quality, the pattern reflective of the increase in microbial load on both treatments of prawns. Prawns were rated with similar sensory quality loss independent of the presence of $ClO_2$.

*Figure 7*

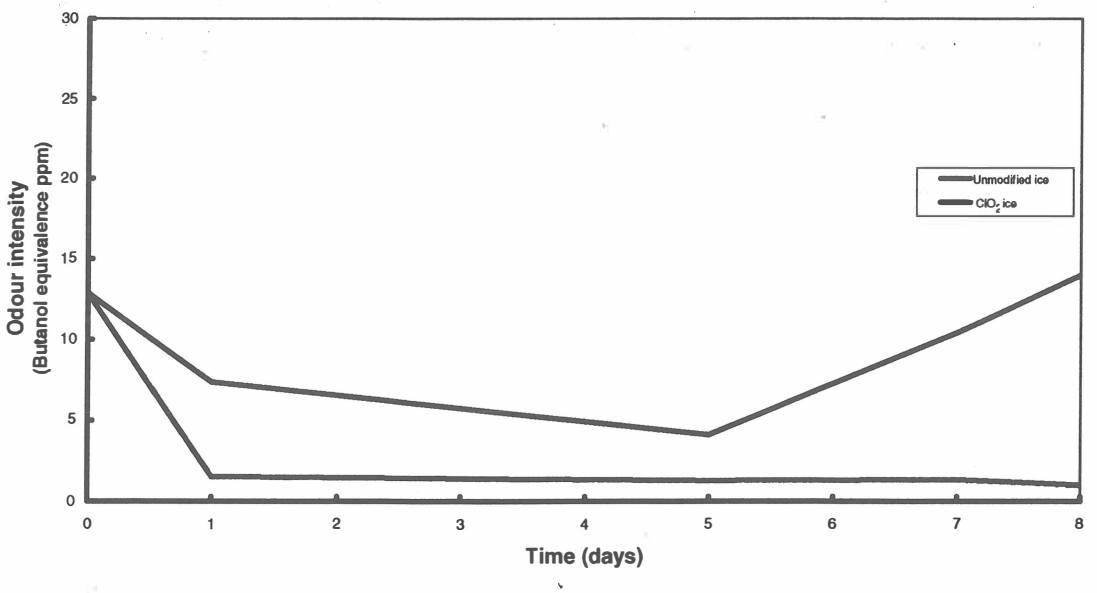
**Sensory testing of cooked tiger prawns**




It is clear from Figure 8 that the presence of $ClO_2$ eliminates the odour of cooked prawns during storage in ice for at least 8 days. This is significantly different from the cooked prawns stored in unmodified ice which exhibited the typical spoilage pattern of loss of odour followed by a steady increase in odour intensity after 5 days storage.

*Figure 8*

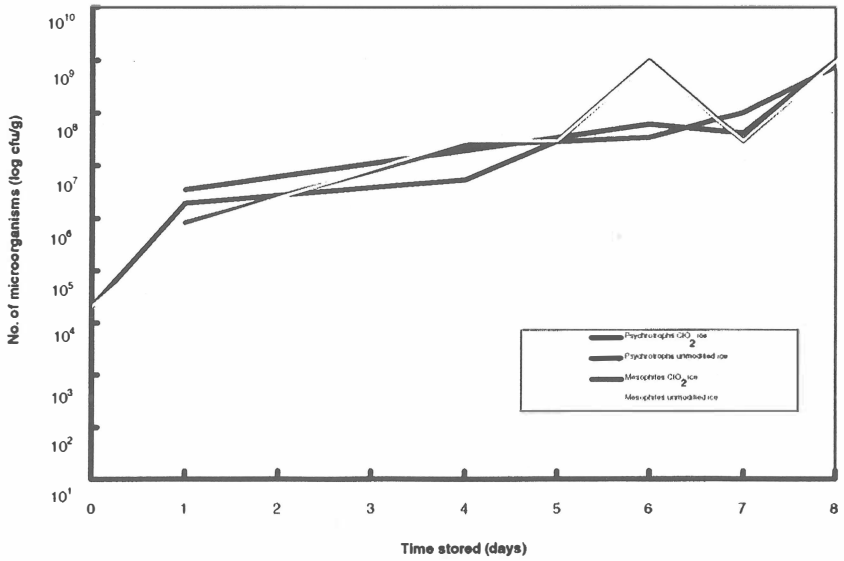
**Odour intensity for cooked tiger prawns**




WHOLE WHITING

The whiting purchased were deemed to be fresh that morning and this appeared to be confirmed by the initial low counts of bacteria present on them. The microbial loads increased with time following an expected and typical pattern (Figure 9). Throughout storage, microbial numbers were similar for both storage treatments, in $ClO_2$ ice and those in unmodified ice.

*Figure* 9 **Microbial load of whiting**




This finding is contrary to the reduced microbial levels on prawns stored in the presence of $ClO_2$. It is unlikely that the response is due to different types of micro-organisms present on the fish from those species present on the cooked Bay prawns or the green tigers, although bacterial species were not identified within this research. The explanation for whiting stored in $ClO_2$ showing no reduction in microbial numbers similar to that which occurred with the stored prawns, cannot be suggested from this work.

Sensory ratings demonstrated that quality of the whiting deteriorated rapidly over storage time (Figure 10). The pattern of deterioration was similar for fish stored in both ice treatments, however there was general agreement of comments from the sensory raters that the fish stored in $ClO_2$ ice looked and smelt worse than those stored in unmodified ice. These comments were received consistently over the complete storage period. Specific observations of softer belly areas and increased belly bursting, as well as greater accumulation of gill mucous were noted for the $ClO_2$ fish. Consideration has been given to possible reaction of chlorine ions and/or oxidising effect with some or other fish components which could result in such deleterious effect but no obvious explanation is evident.

In this trial, the worst possible case was tested which is that of gut in whole fish. Quite different results may be obtained with gilled and gutted fish, rinsing the fish prior to storage or when used with fish fillets.

*Figure* 10

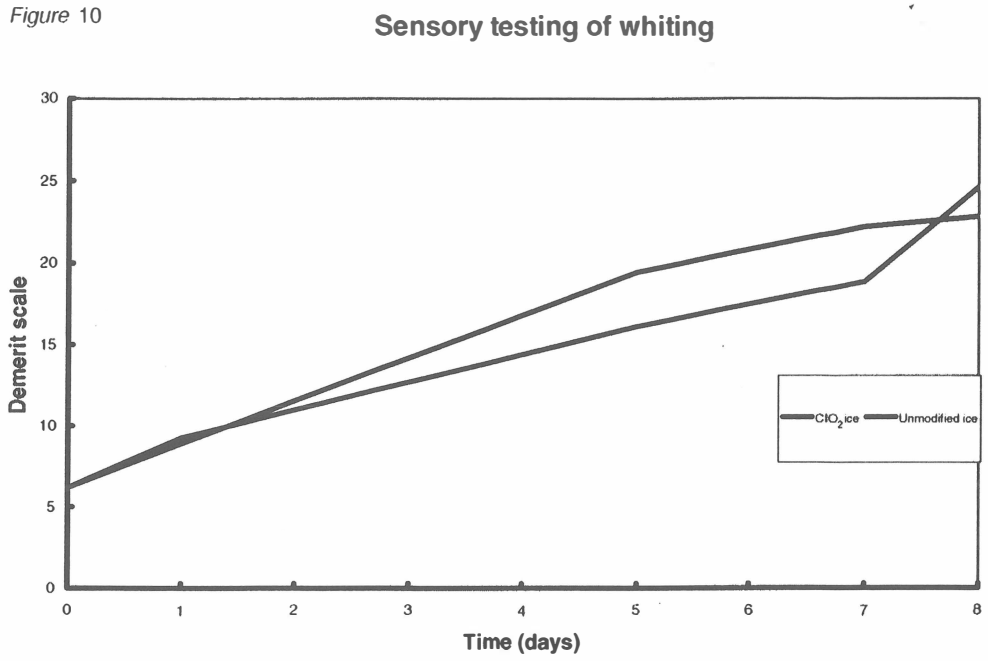
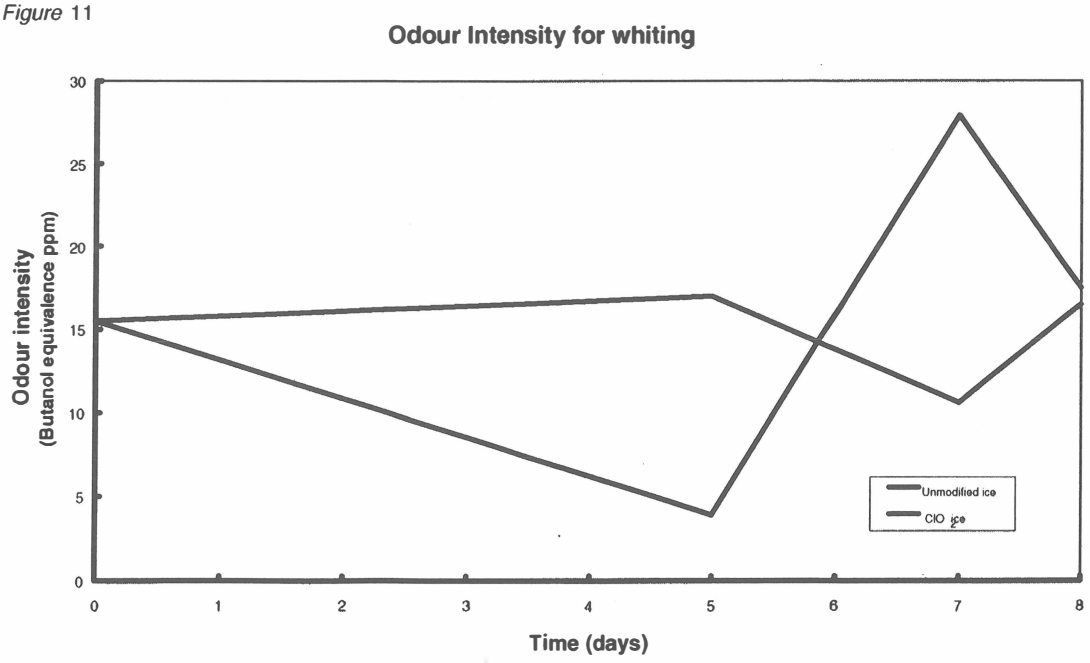



Figure 11 indicates the initial odour intensity of the whiting was high and for those fish stored in unmodified ice the intensity decreased for 5 days, then increased sharply. Unexpectedly the odour intensity of the whiting stored in $ClO_2$ ice did not show the initial loss of odour and the odour intensity remained at a similar level throughout the storage period.

*Figure* 11

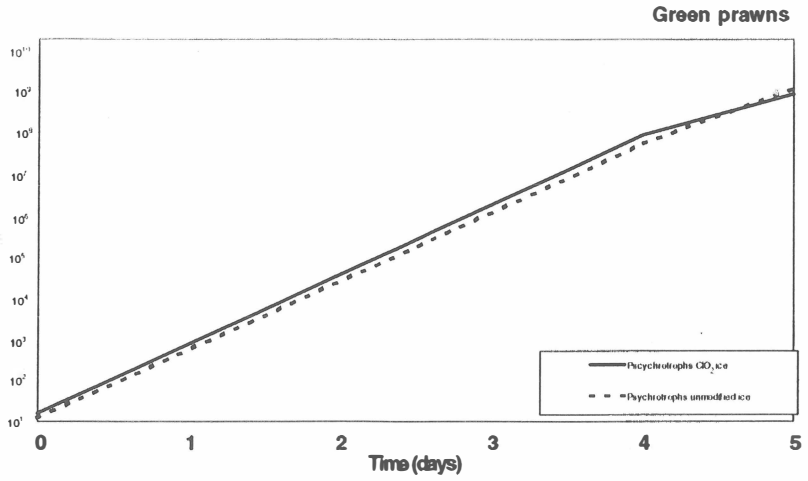


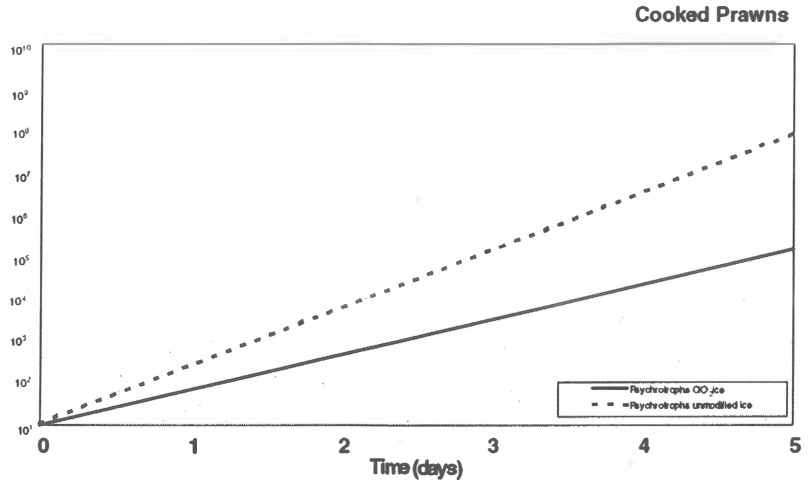
## Dripwater from stored product

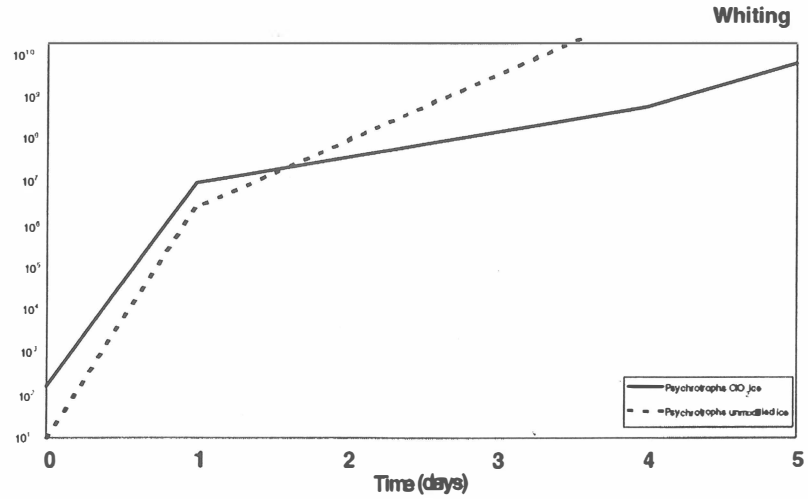
The microbial loads present in the dripwaters from the stored product are depicted in Figure 12, with pschrotrophs only illustrated as these are the most significant organisms with respect to ice. The numbers of micro-organisms present are cumulative from day 0 to day 5 when the dripwater was drained due to storage practicalities. It should be noted therefore, that the results are not directly reflective of the numbers of organisms present on the surface of the product.

In the dripwater from cooked Bay prawns, $ClO_2$ presence appears to retard the accumulation of pschrotrophs indicating a bactericidal effect. A similar effect is observed for dripwater loads from whiting after the first day of storage. With green prawn dripwater, the number of psychrotrophs appears the same for both types of ice. It is possible that the bactericidal effect of $ClO_2$ is limited in this situation because of reaction between $ClO_2$ and phenyloxidase present in green prawns and potentially available in the dripwater, as evidenced in Figure 5.

Figure 12: Microbial load of dripwater



## DISCUSSION

The seafood purchased was assumed to be fresh and of good quality and this was borne out by low initial microbial loads carried on the whiting and green prawns. This point is emphasised as it could have bearing on the effectiveness of $ClO_2$ as a sanitiser. It has been found that the action of many sanitising agents is impaired in the presence of a high organic load (Tanner, 1989) and the higher the organic load, the greater the chlorine demand. With high chlorine demand, bactericidal action competes with chemical reactions for the available chlorine (Tsai *et al.*, 1992) and hence the sanitiser needs to be present in greater concentration to be effective. However, $ClO_2$ demonstrates high efficacy even in the presence of high bacterial load as demonstrated by the effect on the cooked Bay prawns where microbial populations were held at a reduced level despite initially high loads.

**The results show that the overall effect of AquaPlus was to reduce bacterial populations for the seafood products stored in this trial. However, the reduction in microbial numbers was not as great as anticipated or as observed in other overseas trials (pers. comm. Quantum Control, 1998). In this trial, the AquaPlus was incorporated into the ice and therefore direct contact between $ClO_2$ and micro-organisms on the seafood is restricted and dependent upon the ice melting. Additionally, the physical nature of flake ice allows air gaps around the product which reduces direct contact with $ClO_2$ and hence restricts bactericidal action. Despite these limitations, AquaPlus incorporated into ice at 20ppm was effective in reducing both mesophilic and psychrotrophic bacteria.**

Aquaplus incorporated into ice at 20ppm had little or no effect on the physical sensory parameters of either of the prawns tested. Unexpectedly, the whole whiting stored in $ClO_2$ ice were consistently considered to be of poorer quality than those stored in unmodified ice throughout the trial. No obvious explanation for this finding occurs from the work in this investigation.

Intensity of odour was greatly reduced and remained lower for longer, in both green tiger prawns and cooked Bay prawns when stored in $ClO_2$ ice. This is most likely due to reduced microbial loads carried by these prawns, resulting in fewer metabolic end-chain compounds produced and less inherent chemical change occurring. With the whole whiting such an effect was not observed and in fact, the odour intensity seemed greater for fish stored in the $ClO_2$ ice. Again, without further investigation, an explanation of this finding cannot be suggested.

## CONCLUSIONS

Stabilised chlorine dioxide, marketed as AquaPlus, is effective in reducing microbial loads of stored seafoods when incorporated into ice at a concentration of 20ppm. From the findings of this work it is suggested that for product being stored with ice incorporating AquaPlus that increasing the concentration up to 50ppm may provide greater benefit. It is also worth considering the use of crushed ice, where applicable, to allow greater direct contact of $ClO_2$ with product surface.

Ice incorporating AquaPlus was very effective in reducing odour of prawns and therefore suggests a benefit for the storage of these prawns However, whole whiting stored in $ClO_2$ ice did not demonstrate a reduction in odour intensity and quality loss with respect to physical parameters was not retarded either. It appears that whole fish present a different set of factors, perhaps related to different compounds in the fish slime and/or different enzymes present, compared to prawns and $ClO_2$ is reacting in an unknown way. Further investigation in this area is warranted to establish what is occurring.

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