Final Report

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Shelf life study for Vacuum Packed Barramundi fillets

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SEAFOOD SERVICES
AUSTRALIA

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Report for

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by
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CENTRE FOR **FOOD TECHNOLOGY**

Queensland Government
Department of **Primary Industries**

OBJECTIVES

- Establish the storage life of barramundi fillets treated with stabilised chlorine dioxide (zydox ®) and vacuum packed

- Determine the packaging options to meet EU export and market distribution requirements

BACKGROUND

Seafarmers is a marketer of quality Australian farmed seafood and has spent the past 12 months researching, developing and test marketing barramundi into the UK and European markets. Seafarmers directors have been working with the Australian Barramundi Farmers Association (ABFA) and government agencies in a concerted plan to develop the industry and plan for expansion.

In an effort to ensure production of consistent high quality product suitable for the export market, Seafarmers have stipulated specific pre- and post-harvest protocols that growers/suppliers must use within their operation. A complete HACCP/QA Approved Supplier Program, certified by SGS to international standards, has been implemented through all suppliers as this is a prerequisite for export to the EU.

Fresh chilled barramundi is not a cheap fish to grow and market in Australia and is positioned at the top of the market, both here and in the UK. Because of this positioning, it supplies niche markets which have been identified as the food service sector and one chain of exclusive supermarkets. The UK imports most of the seafood it consumes and has supplies from all over the world. Our product must reach the UK market at a price competitive with other quality species.

Our market research program has demonstrated that shipping whole fish to the UK and EU is uneconomic due to high freight costs. The UK market is orientated towards value added product and is extremely quality and price sensitive. It would be beneficial to the growing aquaculture industry to gain the economic return from value adding in Australia.

A recent visit to the UK has identified a number of potential distributors interested in long-term supply contracts providing we meet certain criteria. These include meeting quality parameters, reducing freight costs, satisfactory processing in Australia, consistent shelf life of 7-10 days and servicing a 5 year supply contract.

This is very exciting for the industry, as it is poised to increase production, but the domestic market is already saturated and prices will indeed fall dramatically should further large quantities be put into this market. Export is the only viable alternative for the barramundi industry to increase production.

Satisfactory shelf life needs to be confirmed using barramundi fillet packed as required by the customer. We must offer documentary proof to the customer to be able to enter into a supply agreement.

METHODOLOGY

Processing

Barramundi, 2-4kg whole weight, were harvested from a Northern Territory farm according to Seafarmers documented quality system. Fish were chilled, packed and flown directly to Brisbane. Filleting occurred in a registered seafood export premises and was carried out within 24h of fish harvest. Standard operating procedures of the factory were followed during the filleting process, except for those fillets that were dipped for 2min in an ice slurry with 100ppm activated zydox® (containing 4% chlorine dioxide) prior to packaging.

Packaging

Fillets were packaged in 2kg quantities as follows:

1. No use of chlorine dioxide, tray vacuum packed
2. Chlorine dioxide dip, tray vacuum packed
3. Chlorine dioxide dip, bag vacuum packed
4. Chlorine dioxide dip, tray vacuum packed & inclusion of ClO_2 generating sachets

Tray packs included drip absorbent pads, the bag pack did not. Two ClO_2 generating sachets were included in pack type 4.

Storage

Packs were held chilled, transported to CFT premises and stored in a cold room running at 4^0C. The cold room temperature was monitored on a fixed room logger, as well as a transportable data logger placed inside the room adjacent to the stored packs.

Times of sampling

Random packs were sampled from each treatment at 0 7 10 and 14 days storage.

Physical assessment

At each sampling time, packs for analysis were assessed visually for integrity and driploss; then opened and the initial odour emanating noted. During sub-sampling for analysis, visual quality parameters were assessed and odour of the fillets were recorded.

Microbiological analysis

Sub-samples of four individual fillets were taken aseptically and a composite sample created. The composite sample was diluted 1:10 with sterile 0.1% peptone and thoroughly homogenised for 1min in a Seward 400 stomacher.

Further dilutions were made in 0.1% peptone as appropriate for plating out on specific agar media as detailed below for the enumeration of particular micro-organisms. All media and methods used were as per Australian Standards for the microbiological analysis of foods.

- **Total bacteria**
  Enumerated on Nutrient Agar (NA) after incubation at 30°C for 3 days.

- **Psychrotrophic bacteria**
  Enumerated on Nutrient Agar (NA) after incubation at 4°C for 10 days. These incubation conditions allow growth of those organisms which can grow at low temperatures and hence the count is indicative of those bacterial species likely to be involved in spoilage of the product.

- **Sulphide producing bacteria**
  Enumerated using Iron Agar (Lyngby) (IAL) overlayed and incubated at 30°C for 5 days. These bacteria are frequently associated with the spoilage of seafoods.

- ***Vibrio* species**
  Total *Vibrio* spp present was determined on Thiosulphate Citrate Bilesalts Agar (TCBS) incubated at 35°C overnight (18h). Species of particular interest are *V.parahaemolyticus*, *V.cholerae* and *V.fluvialus*. These species grow with specific typical morphology on TCBS and if suspected, further tests are conducted.

- ***Listeria* species**
  Of specific interest is *L.. monocytogenes* and its presence was determined by a 2-step enrichment in Fraser broth (30°C/24h and 37°C/48h), followed by growth on Oxford and PALCAM agars (37°C / 48h). Agar plates were examined for typical colonies. Due to the enrichment step in this test, results are expressed as present or absent and are not a count of *Listeria* present.

RESULTS

Physical Assessment

The fish fillets were in excellent condition at the start of the storage period, showing all the attributes of fresh high quality product. After 7 days storage at 4°C, the fillet flesh still retained characteristic translucency, sheen and colour. There were no mal-odours present and, in fact, only slight loss of characteristic barramundi fillet odour. (see Appendix 1 for complete descriptions). The retention of characteristic sensory attributes at day 7 of storage was indicative of fillets still in good condition with the only indication of shortened storage being the loss of odour.

At day 10 of storage, the visual appearance of the fillets was acceptable however distinct mal-odours were evident. These differed between packs but were frequently of the 'fruity / sour / metallic-lactate' type and slight 'sulphidey' odours were noted.

After 14 days of storage, the fillets smelt distinctly 'off' with strong 'rotten / sulphide'' odours present.

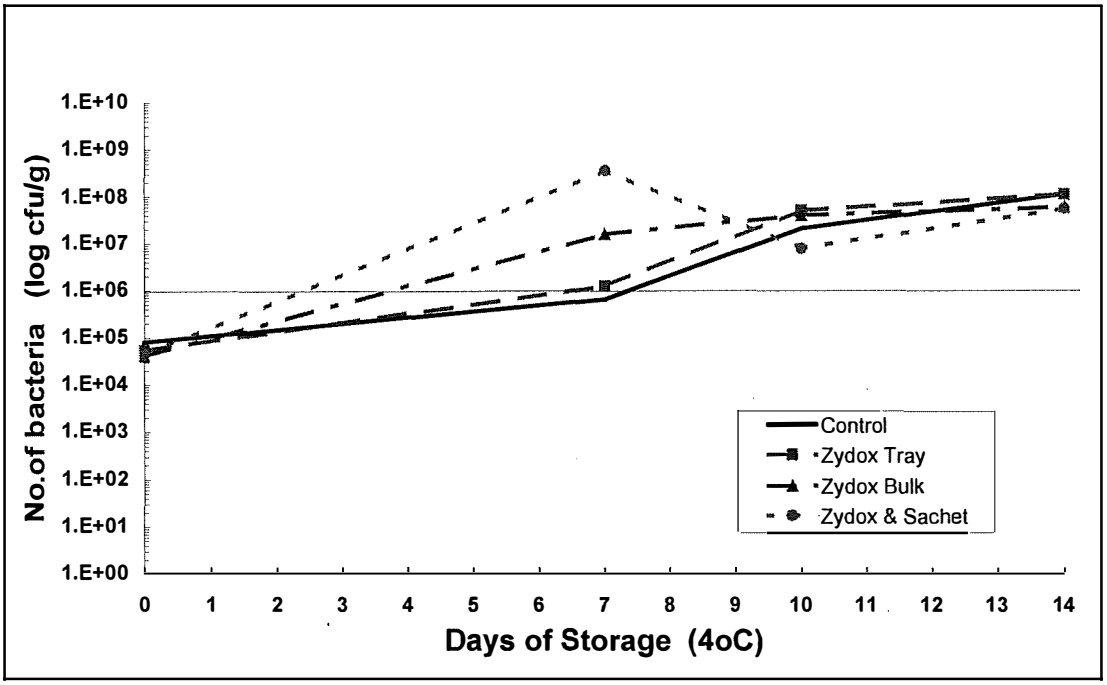
The fillet flesh remained acceptable in appearance, only exhibiting the first signs of discolouration / oxidation after 10 days of storage at 4°C. At no time throughout the storage period was there evidence of slime accumulation on the fillets as is commonly observed with stored fillets of many fish species.

Microbial growth

Total microbial load

The initial microbial load on the barramundi fillets (day 0) was typical of commercially processed fillets. However, to ensure optimal storage life of most fish fillets, initial total microbial load needs to be down around the 10^3/g level (Slattery, unpublished data). To achieve maximum benefit from vacuum packaging and modified atmosphere techniques, it is our experience that microbial loads on seafood should be no more than 10^2-10^3/g.

Figure 1. **Total microbial load on barramundi fillets**



** 1.E in the Figure refers to a log_{10} number, hence 1.E+05 means 10^5 bacteria/g or 100 000 bacteria/g*

Unexpectedly, results show (Figure1) that there was little difference in microbial loads between the untreated fillets and the chlorine dioxide dipped fillets. This was true for all the packaging treatments. Given the initial microbial load on the fish fillets, it could be expected that chlorine dioxide treatment of 100ppm Zydox for 2 minutes would reduce the load by 2 logs, down to the 10^2-10^3/g level. This did not occur in this trial.

Throughout storage there was a normal increase in the microbial population, at a rate typical of the load levels present. The high count obtained from the analysis of the dipped fillets tray-packed with satchets after 7 days storage is likely to be due to the observed poor vacuum present in the packs sampled and therefore is a misrepresentative result. However such an explanation is not valid for the high count obtained from the 'bulk bag' pack as the package was completely intact. Results, in this instance, are illustrative of the variation between fillets and therefore between packs. This inherent variability between fillets and packs has large commercial

implication, demonstrating a need to develop processing procedures which allow leeway in storage life limit for the product.

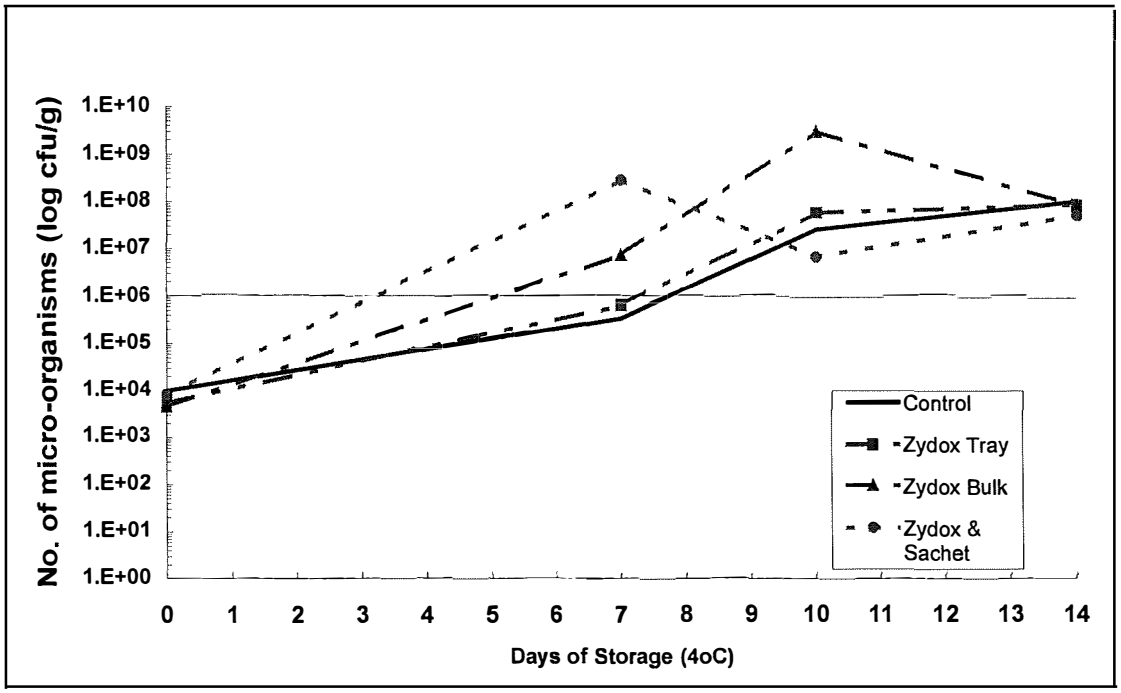
From the results, chlorine dioxide treatment did not appear to have any delayed retardation effect on bacterial growth from any residual present. Additionally, the fillet packs which contained chlorine dioxide generating satchets did not show greatly retarded microbial growth, although population growth appeared to be held from 7 days storage to 10 days storage. This needs to be further investigated and far more data gained to determine the validity of the effect.

Psychrotrophic load

Additional to the contract work specified, psychrotroph counts were also undertaken in addition to the total bacteria present. Psychrotrophs are those bacteria which are capable of growing, if slowly, at low (chill) temperatures and are frequently the organisms involved in spoilage of seafoods. Hence, the numbers of these bacteria present tends to dictate the storage life of the product.

For the fillets in this trial, the level of psychrotroph bacteria present initially was around 1 log (10-fold) less than the total number of bacteria present (Figure 2). However, the rate of growth was rapid and similar to that of the total bacterial population.

Figure 2. **Psychrotrophic load on barramundi fillets**



** 1.E in the Figure refers to a log_{10} number, hence 1.E+05 means 10^5 bacteria/g or 100 000 bacteria/g*

Comparison of Figures 1 and 2 indicates that after 5-7 days storage, psychrotrophs made up the total population present and they had reached levels indicative of no further storage life of the fillets remaining. Again, similar to results for total bacteria

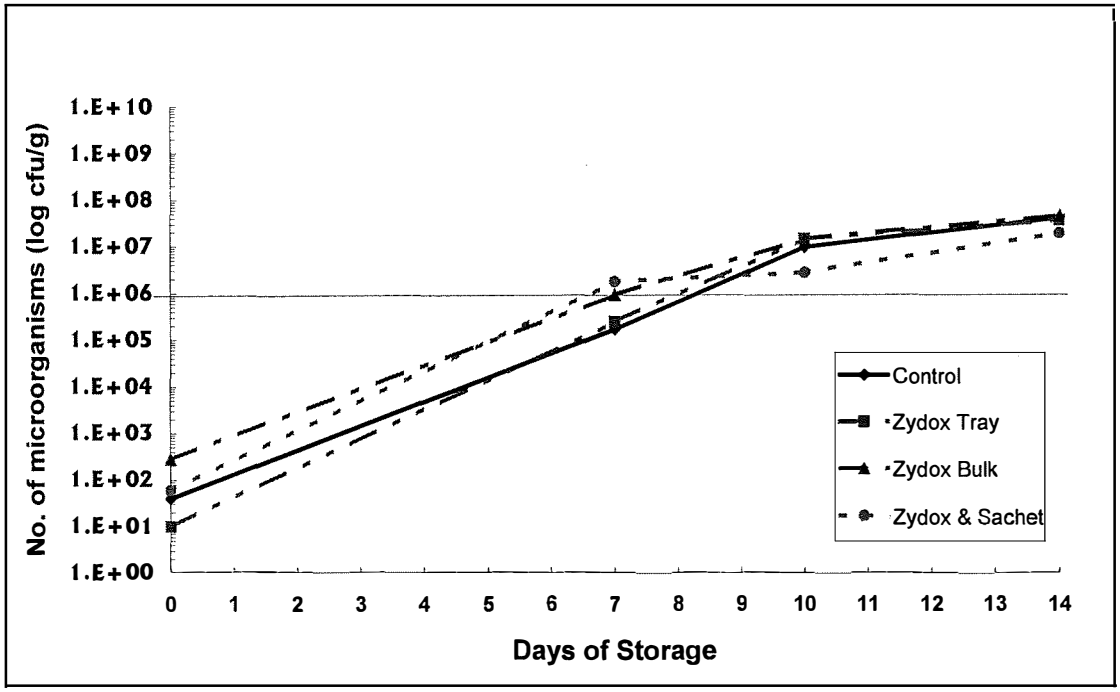
present, it is likely that the high count obtained for the tray packed fillets with satchet present originated from poor or broken vacuum in the packs.

Sulphide-producing bacteria

Those bacteria which are able to produce sulphide compounds from metabolising proteins are frequently common in aquatic environments, therefore are often part of the commensal flora present on fish. Typically, many of these bacterial species are also able to grow happily at low temperatures.

Figure 3 shows that initial numbers of these bacteria on the fillets were low, but increased rapidly over 7 days storage to reach numbers where their metabolic byproducts would be accumulating noticeably. When present at levels $>10^6$/g, spoilage odours and flavours would be increasingly noticeable.

Figure 3. **Sulphide-producing bacteria on barramundi fillets**



** 1.E in the Figure refers to a log_{10} number, hence 1.E+05 means 10^5 bacteria/g or 100 000 bacteria/g*

The rapid growth of sulphide producing bacteria is frequently observed with vacuum-packed product as the organisms are facultative anaerobes and hence grow very readily in atmospheres of low oxygen. With this metabolic ability, these bacteria can compete and outgrow other species present. Many species of these bacteria grow on iron agar with a very typical pinkish/brown colony colour. Such colony types were observed to comprise around 50% of the bacterial population on the Day 7 samples.

***Vibrio* species**

There was no growth evident on any of the TCBS plates (which isolate *Vibrio* species) from any sample of any of the packaging treatments. As 1 ml of the composite sample was plated in total, this result implies a *Vibrio* spp count of < 10/g.

***Listeria* species**
Listeria was absent from all samples. This held true throughout the storage period.

DISCUSSION

For Australia to supply the European market with fresh chilled farmed barramundi fillets, a minimum storage life of 10 days is required to allow for transport and wholesale/retail distribution within the market. Such a storage time is beyond that obtainable through normal processing /handling techniques, as bacterial growth on the product would cause spoilage before 10 days. Hence hurdle technology which uses several methods of retarding spoilage, is needed to reduce the bacterial load and restrict the growth of the microbial population present.

The market demand for fresh chilled fish fillets dictates that any processing technique used to extend the storage life results in minimal changes to the product. It was considered that reducing the microbial load on the fish fillets after processing with chlorine dioxide and packing the fillets under vacuum should work adequately together to limit the growth of bacteria and hence extend storage life of the fillets. It was possible that the extension attainable would be to 10-12 days although recognised that this storage life would be on the limit of that achievable.

The results indicate that chilled vacuum-packed barramundi fillets, processed and handled under good manufacturing standard operating procedures and with the addition of a chlorine dioxide dip prior to packaging, have a storage life of 7 days. This was disappointing and is similar to that expected with straight vacuum-packed chilled fillets. It appears the chlorine dioxide dip provided little benefit in reducing the bacterial population present on the fillets, as evidenced by the bacteria enumerated at the start of the trial. However it needs to be kept in mind that the chlorine dioxide concentration used in the fillet dip was reasonably, low providing only 4ppm at activation.

This finding was unexpected and no ready explanation is obvious. Several reasons could be applicable. The concentration of the chlorine dioxide dip was insufficient; the contact time between chlorine dioxide and bacteria was not long enough; the activation of the chlorine dioxide solution was inappropriate for the operational procedure or the time delay between activation of the ClO_2solution and fish contact was too long.

It is considered that all of these reasons could have been involved to varying extents and further work is required to establish practicable operational procedures to gain maximum effect from chlorine dioxide in reducing bacterial loads. It is suggested that a further trial be conducted, under similar conditions, but using a higher concentration of chlorine dioxide for a longer contact period with activation of the ClO_2 occurring concurrently with dipping of the fillets.