NUTRITIONAL PROFILES OF BAITFISH 3: EFFECTS OF HARVEST AND POST-HARVEST PROCESSES ON QUALITY OF LOCAL BAITFISH FOR FEEDING SBT

Richard Musgrove, John Carragher, Andy Manning, Ben Zammit, Philip Thomas and Jeff Buchanan

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PRINCIPAL INVESTIGATOR: Dr John Carragher

ADDRESS: SARDI Food Value Chain
          PO Box 120
          Henley Beach
          SA 5022

OBJECTIVES:

1. To survey the consequences of existing harvest and post-harvest handling practices on measures of nutritional quality of local baitfish for feeding tuna.

2. To determine the effects of different harvest and post-harvest handling practices on measures of nutritional quality of local baitfish for feeding tuna.

3. To make recommendations to industry (baitfish fishers and tuna farmers) regarding existing or modified harvest and post-harvest handling practices that maximise the nutritional quality of local baitfish for feeding tuna.
OUTCOMES ACHIEVED
The research documented in this report demonstrates the effects of post-harvest practices on the nutritional quality of locally-caught Australian sardines (*Sardinops sagax*) and of redbait (*Emmelichthys nitidus nitidus*) sourced from Tasmania. The research showed that there were substantial losses in key elements of the nutritional profile (i.e. vitamins and nucleotides), concomitant with increases in rancidity and loss of freshness. Greatest losses in quality and freshness occurred during post-harvest transport and freezing, and thawing of baitfish, prior to feeding to SBT. Losses during extended frozen storage can be substantial.

This information will be used by suppliers to improve post-harvest treatment of baitfish to optimise quality. The tuna industry will use the information to improve the efficiency and effectiveness of their tuna-feeding strategy to optimise SBT growth performance, health, flesh quality and return at the market.

NON TECHNICAL SUMMARY
Past SBT Aquaculture Subprogram-Aquafin CRC projects have sought to determine the nutritional profiles of the 23 or so different baitfish species that are used by tuna farming companies to feed to southern bluefin tuna (SBT). These studies have shown inter- and intra-species differences in nutritional parameters including amino acid and fatty acid ratios, crude protein, crude fat, ash and energy, and indicators of biochemical quality, such as free fatty acids and peroxide levels. Whilst differences in these characteristics between species are expected, differences between different batches of the same species can indicate either seasonal or regional effects, and/or the impacts of different harvest and post-harvest practices on baitfish quality. For example, data were collected from 34 different batches of local sardines supplied to the tuna farms. Whilst some proximate parameters such as protein vary only slightly (10% or less), the level of fat changes over 6-fold, and that of peroxides over 10-fold between batches.
Not only does this indicate that it is important to know when to harvest the sardines in order to maximise their nutritional value (i.e. when fat levels are high before spawning), it also shows the effects of oxidative processes (e.g. lack of antioxidants, prolonged processing and/or storage times, inadequate storage temperatures) on the quality of the nutrients (especially the fats) in the baitfish. In this regard there was a significant positive correlation ($R^2 = 0.61$) between storage time and peroxide concentration, suggesting that the storage conditions in Port Lincoln (South Australia) can lead to high peroxide levels in the local sardines (Ellis and Rough, 2005).

Tissue integrity and health of the SBT is damaged by oxidative processes and protected by antioxidant vitamins. These protective antioxidants have to be supplied to tuna in their feed. High peroxide values are of concern because they indicate low levels of antioxidant vitamins in baitfish, and that the tuna consuming the baitfish are taking in a high burden of reactive oxygen species that can lead to further antioxidant depletion and peroxidation of the structural and functional compounds. Both mechanisms (i.e. low vitamins or high oxidative burden) could, in turn affect metabolic processes, and impact on flesh quality characteristics. It will be important to be aware of the potential deleterious effects of long-term feeding of nutritionally poor-quality baitfish on the health and flesh quality of tuna.

Postharvest deterioration in Australian sardine (*Sardinops sagax*) nutritional quality was followed from the jetty through short (4-6 weeks) and long (3-6 months) term storage trials (Objective 1) to subsequent thawing (Objective 2) for feeding to SBT. Samples were taken from fishing vessels at the jetty and during and after storage and thawing trials. Redbait (*Emmelichthys nitidus nitidus*) were sampled directly after capture onboard the fishing vessel, at the Triabunna (Tasmania) factory, then at the Port Lincoln factory/commercial freezer during and after storage and thawing trials.

The results of this study are as follows:

- Existing harvest and post-harvest practices have a significant impact on the nutritional quality of baitfish fed to SBT in seacages. Vitamins and nucleotides decline to low levels, rancidity increases and fish
• Freezing method affected the rate of vitamin E loss. IQF sardines stored at -20°C lost vitamin E at a higher rate than those which were block frozen, possibly because individually frozen fish are more likely to be exposed to air than those in a solid block.

• Baitfish, particularly sardines, should be thawed in seawater, not air, to minimise vitamin losses. Vitamin E losses were actually least in freshwater and greatest in air, whereas for vitamin C this was reversed in some trials. The seawater recommendation is a compromise to minimise losses of both vitamins. Air-thawing, particularly at room temperature, accelerates oxidative processes and thus irreversible breakdown of antioxidants within fish tissue.

• Block feeding at sea is recommended over land-based thawing as rapid thawing (1-2 hours vs 1-2 days) may reduce vitamin losses.

• In addition, fish should be kept on ice or refrigerated when transferred from the fishing vessel’s refrigerated seawater tanks to the factory, and storage time limited.

• Good correlations between TBARS and remaining vitamin E suggest a practical utility of this measure as an indicator of antioxidant status, with 1-1.5mg/kg TBARS equating to 50% remaining vitamin E in each baitfish species. In view of this result it is recommended that TBARS be included in as an analytical requirement for baitfish quality analysis in Port Lincoln. Analysis of TBARS is also methodologically simpler and less time-consuming than for K factor.

Objective 3 was addressed in consultation with the ASBTIA. It was decided that the most effective means of industry extension would be the production of a laminated summary of the key results for distribution to sardine fishers, processors and tuna farm operators. This was carried out. Key results were also included in industry newsletters (Tuna Briefs; http://www.sardi.sa.gov.au/pages/aquafin/southern_bluefin_tuna_publications.htm?sectID=967&tempID=11#Newsletters) and discussed with industry at SBT.
Aquaculture Subprogram-Aquafin CRC meetings at Port Lincoln. Results were also presented at the Aquafin CRC conference in the Barossa Valley, May 2007.

KEYWORDS: baitfish, sardines, harvest, post-harvest, quality, vitamins, ice hours.

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BACKGROUND
The bulk of the South Australian sardine (Sardinops sagax) Total Allowable Catch (TAC; 25,463 tonnes in 2006) is sold to southern bluefin tuna (SBT) farmers as feed for their fish. 70 to 80% of these sardines are delivered to the tuna feed boats as a fresh chilled product, with the remainder frozen on-shore, and stored in freezers either short-term or long-term before distribution to tuna farms.

Although the existing sardine fishing fleet and tuna aquaculture industry are both based at Port Lincoln (South Australia), the main sardine fishing grounds are 12-24 hours steaming from the tuna pontoons and on-land freezers. This time delay, together with the limited capacity of most existing sardine boats to handle the pursed sardines in their chilled brine tanks, means that the
particularly fragile sardines are often physically damaged when they are finally fed to the tuna or frozen. This physical deterioration is mainly evident as scale loss, bent and twisted bodies, an overall softening of the flesh, blood and moisture loss and bursting of the belly. As well as causing physical deterioration, current harvest and post-harvest processes are almost certainly affecting the biochemical properties of the sardines that, in turn, could be reducing the nutritional quality and/or palatability of this local baitfish for consumption by tuna.

Redbait (*Emmelichthys nitidus nitidus*) is also used by the tuna farming industry to supply a higher (2-5 times) level of fat and energy than local sardines. The quantity of redbait used by the tuna industry is 5,000 tonnes p.a. but there is potential to access another 20,000 tonnes of quota. The redbait is caught off the east coast of Tasmania by a medium-sized vessel using a midwater trawl and the fish are transferred to chilled seawater holding tanks until the vessel returns to Triabunna (1-4 days later) and the catch of several hundred tonnes is transferred to the on-land freezing works. There they are frozen into 20kg blocks, a process that takes several days to complete. Orders for redbait are then dispatched in freezer containers to Port Lincoln where they may be stored for several months before being thawed and fed to the tuna. Although redbait is reportedly tougher and so less easily damaged than the sardine, postharvest nutritional degradation is critical particularly as fat levels can be up to 10 - 12%, and fat is the component most likely to spoil.

The aim of this project was to better understand the factors that affect the nutritional quality of locally caught baitfish (sardines and redbait) for the feeding of farmed tuna. The effects of season, handling practices and processes (ie time-temperature characteristics), freezing method (ie blocks, IQF), storage temperature and storage duration on a number of nutritional quality parameters have been determined.

**NEED**

Past SBT Aquaculture Subprogram-Aquafin CRC projects have sought to
determine the nutritional profiles of the 23 or so different baitfish species that are used by SBT farming companies to feed to tuna. These studies have shown inter- and intra-species differences in nutritional parameters including amino acid and fatty acid ratios, crude protein, crude fat, ash and energy, and indicators of biochemical quality, such as free fatty acids and peroxide levels. Whilst differences in these characteristics between species are expected, differences between different batches of the same species can indicate either seasonal or regional effects, and/or the impacts of different harvest and post-harvest practices on baitfish quality. For example, data were collected from 34 different batches of local sardines supplied to the tuna farms. Whilst some proximate parameters such as protein vary only slightly (10% or less), the level of fat changes over 6-fold, and that of peroxides over 10-fold between batches.

Not only does this indicate that it is important to know when to harvest the sardines in order to maximise their nutritional value (ie when fat levels are high before spawning), it also shows the effects of oxidative processes (eg lack of antioxidants, prolonged processing and/or storage times, inadequate storage temperatures) on the quality of the nutrients (especially the fats) in the baitfish. In this regard there was a significant positive correlation ($R^2 = 0.61$) between storage time and peroxide concentration, suggesting that the storage conditions in Port Lincoln can lead to higher peroxide levels in the local sardines (Ellis and Rough, 2005).

Tissue integrity and health of the SBT is damaged by oxidative processes and protected by antioxidant vitamins. These protective antioxidants have to be supplied to tuna in their feed. High peroxide values are of concern because they indicate low levels of antioxidant vitamins in baitfish, and that the tuna consuming the baitfish are taking in a high burden of reactive oxygen species that can lead to further antioxidant depletion and peroxidation of the structural and functional compounds. Both mechanisms (i.e. low vitamins or high oxidative burden) could, in turn affect metabolic processes, and impact on flesh quality characteristics. It will be important to be aware of the potential deleterious effects of long-term feeding of nutritionally poor-quality baitfish on
the health and product quality of tuna.

OBJECTIVES

1. To survey the consequences of existing harvest and post-harvest handling practices on measures of nutritional quality of local baitfish for feeding tuna.

2. To determine the effects of different harvest and post-harvest handling practices on measures of nutritional quality of local baitfish for feeding tuna.

3. To make recommendations to industry (baitfish fishers and tuna farmers) regarding existing or modified harvest and post-harvest handling practices that maximise the nutritional quality of local baitfish for feeding tuna.
1. METHODS

Objective 1 was addressed as follows:

A. Fleet surveys. Two sardine fleet surveys were undertaken (November 2005 – March 2006 and March 2006 – June 2006) with fish sampled from boats unloading catches at the Port Lincoln jetty. In each case four replicate samples of 10 whole fresh fish were sampled from each of 12 sardine boats. Samples were frozen in liquid nitrogen, packed in dry ice then transferred to a -80°C freezer until analysed.

B. Storage Trials. Short (4-6 weeks) and long term (3-6 months) storage trials were run using commercial freezers (-18°C). Groups of 20kg blocks of recently frozen sardines were sub-sampled by removing a section with a band-saw at weekly or monthly intervals. Whole redbait were sampled at the net and the Triabunna factory, in each case immediately stored in liquid nitrogen. On reaching Triabunna these samples were transferred to a dry shipper and sent to Port Lincoln for analysis. Frozen 20kg redbait blocks were transported to Port Lincoln and sampled at weekly or monthly intervals from commercial freezers (-18°C). Samples were frozen in liquid nitrogen, packed in dry ice then transferred to a -80°C freezer until analysed as described below. Data loggers were used to track temperatures during transit and storage.

Objective 2 was addressed with a series of thawing trials using both species. Groups of four or six frozen 20kg blocks of sardines or redbait were thawed in saltwater, freshwater or air, either at 4°C or at ambient temperature for 3-4 days with samples taken daily (10 fish/sample). Refrigerated fresh sardines were also run for the same period. In addition, fish from several of the block-thawing trials were subjected to thaw/freeze/thaw regimes within their respective treatments. In each case fish were sampled during the trials for biochemical analysis as described below. Replicates were not run within single trials; however the same experiments were carried out several times. Data loggers were used to track temperatures during thawing and refrigerated storage.
Objective 3 was addressed in consultation with the ASBTIA. It was decided that the most effective means of industry extension would be the production of a laminated summary of the key results for distribution to sardine fishers, processors and tuna farm operators. This was carried out. Key results were also included in industry newsletters (Tuna Briefs; http://www.sardi.sa.gov.au/pages/aquafin/southern_bluefin_tuna_publications.htm?sectID=967&tempID=11#Newsletters) and discussed with industry at SBT Aquaculture Subprogram-Aquafin CRC meetings at Port Lincoln. Results were also presented at the Aquafin CRC conference in the Barossa Valley, May 2007.

A. Analytical methods

Baitfish within each replicate were pooled and minced twice prior to biochemical analysis. Pooled tissue samples were analysed using standard methods to determine the effect of storage and thawing on nutritional quality (i.e. crude fat, moisture, vitamin C and vitamin E) and for evidence of loss in quality (i.e. K Factor, TBARS (thiobarbituric acid reactive substances, measured as mg/kg malonaldehyde), as follows.

Crude fat was determined with ethyl acetate extraction; the method adapted from Norwegian Standard NSF (NS 9402) by Thomas (unpublished). Moisture was determined as follows. Approximately 11-12g of wet pilchard tissue was chopped up finely using a cleaver, allowing enough tissue for two duplicate samples of ~ 5g. 5 grams of tissue was then weighed and added to an aluminium foil pan of known weight. Once all samples were completed, they were then transferred into a preheated Memmert drying oven for 24 hours at 60°C. All samples were then removed, allowed to cool for 5 minutes then weighed to 0.001g.

Vitamin E (α-tocopheryl) concentrations were determined by a HPLC method based on the method of Huo (1999). Vitamin C was determined by a technique based on the HPLC fluorescence detection method of Brown and
Miller (1992) which was adapted to SBT muscle in the laboratory at the Lincoln Marine Science Centre with the cooperation of Malcolm Brown of CSIRO Hobart, Tasmania.

Nucleotides were determined by the HPLC methods of Ryder (1985) and Van der Boon (1985).

K factor was calculated as:

\[ \text{K factor} = \frac{\text{Hx} + \text{Ino}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{Ino} + \text{Hx}} \times 100 \quad (\text{Gill, 1992}) \]

Where: ATP= adenosine triphosphate
ADP= adenosine diphosphate
AMP= adenosine monophosphate
IMP= inosine monophosphate
Ino= inosine
Hx= hypoxanthine

TBARS (thiobarbituric acid reactive substances) were measured spectrophotometrically as mg/kg malonaldehyde (Wong et al., 1991).

Anti-nutritional factors such as histamine, peroxide and lipid hydroperoxides (LPO) were also investigated, however extensive analysis for these produced largely inconclusive and highly variable data/results, with histamine (Neogen: Alert Histamine extraction kit, cat no. 9520) producing the only useful, albeit limited, data. Concomitant testing and modification of the methods failed to resolve issues with the remaining parameters, therefore these data are not included in the following report.

Recommended minimum vitamin inclusion levels (for pellets) were converted to wet weight (sardines and redbait) as follows:

\[ \text{RIL}_F = \frac{\text{RIL}_P}{\left( \frac{\% \text{ dry matter}_P}{\% \text{ dry matter}_F} \right)} \times \frac{1}{\text{Antioxidant}^*} \]
Where: $F =$ fish and $P =$ pellet.

$RIL_P = \text{Minimum recommended vitamin inclusion levels for pellet-fed SBT:}$

45 mg/kg for Vitamin E and 60 mg/kg for Vitamin C (pers. comm. J Buchanan).

$\% \text{ dry matter}_F = 100 - \% \text{ mean moisture level. For sardines mean } \% \text{ moisture}$

was 73.6 + 0.12 (n=290) and for redbait it was 69.1 + 0.14 (n=335).

Synthetic dl-$\alpha$ tocopherol acetate was added to the pellet but endogenous or

native $\alpha$ tocopherol was measured in fish (pers. comm. P Thomas). The

native form of $\alpha$ tocopherol has 1.4 times the activity level of the synthetic

form (Scherf et al., 1996).

Data loggers were used to track temperatures during transport and storage,

and ice hours calculated following Bremner et al (1987) as follows:

Ice hours = rate of deterioration ($r$) x storage time (h).

Where: $r = (1+0.1t)^2$ and $t =$ temperature in °C over a given storage period, in

thus case fractions of an hour; as temperature was taken at 10 minute

intervals.

Ice hours is a defined as equivalent hours of fish storage on ice (Bremner et al., 1987), that is, how the actual storage conditions and their effect on fish deterioration equate to storing the same fish on ice for the same period. Thus

1 hour on ice (at 0°C) is equal to 1 ice hour, the same hour spent at 5°C is equal to 2.25 ice hours, at 10°C this would be the equivalent, in terms of fish deterioration, to spending 4 hours on ice.

**B. Statistical analysis**

Data were analysed with SPSS Version 14.0 modules relating to Curve Estimation, GLM (ANOVA, ANCOVA) and T-tests. Where data could not be normalised, Mann-Whitney U or Kruskel Wallis H tests were used. P was
accepted at 0.05 and data shown as mean ± SE. “Best fit” curves appearing on figures are based on regression equations derived from raw data. Replicates were not run within single thawing trials, limiting analytical options. Results are discussed where trends are similar between repeated experiments.
2. RESULTS

A. Fleet Survey

There were two sardine fleet surveys undertaken; because of fishing patterns each of these has been divided into two groups (Fig 1) with data analysed on the basis of groups, not surveys. Each group of samples took approximately a month to collect and there was at least a month between groups.

i. Vitamins

There were no significant seasonal changes in sardine vitamin C level due to a significant boat x group interaction (P<0.001). Redbait showed a significantly lower vitamin C level (9.84 ± 0.69 mg/kg) than the lowest of the sardine samples (13.50 ± 1.13 mg/kg) (P = 0.033) taken at the same time of year (i.e. mid-January/ early February; sardine group 2, Fig 1a).

![Fig 1a Sardine fleet survey groups](image)

Each point represents a mean (+SE) derived from 4 replicate samples of 10 whole fresh fish.
There were significant seasonal changes in sardine vitamin E level (Fig 1b) with a reduction in March followed by an increase in May (P<0.001). Redbait showed a significantly higher vitamin E level (13.98 ± 0.42 mg/kg) than the highest of the sardine samples (11.35 ± 0.39 mg/kg) (P = 0.004) taken at the same time of year (i.e. mid-January/ early February; sardine group 2, Fig 1b).

Fig 1b Sardine fleet survey groups and redbait factory samples analysed for vitamin E
Each point represents a mean (+SE) derived from 4 replicate samples of 10 whole fresh fish.
ii. TBARS and K factor

There were significant increases in sardine and red bait TBARS during January 2006 (P \leq 0.007) (Fig 1c), indicating an increase in rancidity during summer. There were no significant seasonal changes in sardine or red bait K factor (ANOVA, P > 0.05, Fig 1d).

Fig 1c Sardine fleet survey groups and red bait factory samples analysed for TBARS
Each point represents a mean (+ SE) derived from four replicate samples of 10 whole fresh fish

Fig 1d Sardine fleet survey groups and red bait factory samples analysed for K Factor
Each point represents a mean (+ SE) derived from four replicate samples of 10 whole fresh fish
iii. % Crude Fat

There were no significant seasonal effects on crude fat level on a fleet wide basis. Redbait showed significantly higher % crude fat (9.44 ± 0.20%) than the highest of the sardine samples (2.42 ± 0.27%) (P < 0.001) taken at the same time of year (i.e. mid-January/ early February; sardine group 2, Fig 1e).

Fig 1e Sardine fleet survey groups 1 2 3 4 and redbait factory samples * analysed for % crude fat
Each point represents a mean (+ SE) derived from four replicate samples of 10 whole fresh fish

Percent crude fat did not change in the following storage and thawing trials so is not considered further in this section.
B. Storage

i. Vitamins

Frozen storage had an effect on the levels of both vitamins (Figs 2 & 3, Tables 1 & 2). Sardine vitamin C declined 29% during short term frozen storage but no trend was detectable in long term storage. Redbait vitamin C declined by approximately 23% over 9 weeks and 60% over 3.6 months (102 days). The apparent increase to 6 months probably has little biological significance, being more a reflection of the analysis of a heterogeneous material, that is, where change in quality over time might vary within a given block or mixing was incomplete within a given sample.
Fig 2 Sardines and redbait: short and long term storage effects on vitamin C (mean mg/kg ±SE)

- **Sardines**
  - Short Term Block
  - Long Term Block

- **Redbait**
  - Short Term Block
  - Long Term Block

**Variables**
- Vitamin C (mg/kg)
- Days of storage
- Storage conditions:
  - Frozen storage
  - Frozen Transport Triabunna to Port Lincoln
  - Factory freezing
  - Net/Jetty
Vitamin E declined markedly during frozen storage of both sardines and redbait (Fig 3, Tables 1 & 2). In most cases initial levels were high but declined rapidly during storage (Table 2), with total losses up to 84% and 94% for sardines and redbait respectively. There were no significant differences between % losses in sardines and redbait in the block storage trials (ANOVA, P=0.095), but over 6 months redbait lost significantly more vitamin E than sardines (P=0.002). In order to standardise time, the short-term storage comparison was made using the 6 week data for both species. The 9 week
data were not included in analysis because, as indicated previously, the observed increase may have more to do with biological heterogeneity of the sample than actual trends during storage.

IQF sardines had higher initial vitamin E levels (IQF Mean = 14.1 ± 0.76mg/kg; Block-frozen mean of 6 wk = 9.46 ± 0.38mg/kg, mean of 6 month = 7.74 ± 0.22; P<0.004, T Test) but by the end of the trials these were very similar to those in blocks. Overall, IQF sardines lost more vitamin E than those which were block frozen during both long (Mann Whitney U, P = 0.021) and short term (P = 0.021) storage (Table 2). The greatest change occurred during the first few days of the storage period (i.e. jetty to freezer). Although the correlation coefficients were high (Table 1), the curve and the calculated values are approximations only; actual fine-scale (i.e. day to day) losses were not measured.

Table 1 Vitamin C and E regression statistics for short (6 - 9wk) and long (6mo) term storage for sardines and redbait.

All regressions significant (P = 0.002 to <0.001)
wk = weeks, mo = months. * Redbait were held for 9 weeks. ^Correlation coefficient for storage period of 3.6 months

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Species</th>
<th>Frozen form</th>
<th>R Square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6wk</td>
<td>6mo</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Sardines</td>
<td>Block</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Redbait</td>
<td>Block</td>
<td>0.56*</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Sardines</td>
<td>IQF</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Block</td>
<td></td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Redbait</td>
<td>Block</td>
<td>0.74</td>
</tr>
</tbody>
</table>
Table 2 Vitamin E and C: % losses during short (6 – 9wk) and long term storage (6mo) of block-frozen or IQF fish in a commercial freezer at -18°C

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Storage period</th>
<th>Block/IQF</th>
<th>% Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sardines</td>
<td>6 wk</td>
<td>block</td>
<td>29</td>
</tr>
<tr>
<td>Redbait</td>
<td>9 wk</td>
<td>&quot;</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>3.6 mo</td>
<td>&quot;</td>
<td>60</td>
</tr>
</tbody>
</table>

B) Vitamin E

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Storage period</th>
<th>Block/IQF</th>
<th>% Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sardines</td>
<td>6 wk</td>
<td>IQF</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>block</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>6 mo</td>
<td>IQF</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>block</td>
<td>56</td>
</tr>
<tr>
<td>Redbait</td>
<td>6 wk</td>
<td>&quot;</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>6 mo</td>
<td>&quot;</td>
<td>94</td>
</tr>
</tbody>
</table>
ii. **TBARS and Nucleotides**

TBARS and K factor (Figs 4 & 5, Table 3) increased during storage for both sardines and redbait (Table 3). Redbait showed the greatest increase in TBARS, particularly during long-term storage (22 fold increase) and in K factor during short-term storage (6.5 fold). TBARS and K factor in IQF sardines rose significantly more than in block-frozen fish during long term storage ($P = 0.007$ and $0.013$ respectively, T Test). Both parameters increased for redbait more than sardines ($P<0.001$) in long term storage but only K factor showed this effect in short term storage ($P<0.001$).

**Fig 4 Sardines and redbait: short and long term storage effects on TBARS (mean mg/kg ±SE)**

![Graph showing TBARS (mg/kg) vs Days of storage for Sardines and Redbait in Short Term and Long Term IQF and Block storage](image-url)

- **Sardines**
  - Short Term IQF
  - Long Term IQF
  - Short Term Block
  - Long Term Block

- **Redbait**
  - Short Term Block
  - Long Term Block
Fig 5 Sardines and redbait: short and long term storage effects on K Factor

**Sardines**
- Short Term IQF
- Long Term IQF
- Short Term Block
- Long Term Block

**Redbait**
- Short Term Block
- Long Term Block

Days of storage

K Factor %
Table 3 K factor and TBARS regression statistics for short (6 - 9 wk) and long (6 mo) term storage for sardines and redbait

All regressions significant (P = 0.002 to <0.001). wk = weeks, mo = months. *Redbait short-term trials were run for 9 weeks.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Species</th>
<th>Frozen form</th>
<th>6 wk</th>
<th>6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>K Factor</td>
<td>Sardines</td>
<td>IQF</td>
<td>0.572</td>
<td>0.979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>block</td>
<td>0.505</td>
<td>0.973</td>
</tr>
<tr>
<td></td>
<td>Redbait</td>
<td>block</td>
<td>0.841*</td>
<td>0.828</td>
</tr>
<tr>
<td>TBARS</td>
<td>Sardines</td>
<td>IQF</td>
<td>0.658</td>
<td>0.586</td>
</tr>
<tr>
<td></td>
<td></td>
<td>block</td>
<td>0.615</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Redbait</td>
<td>block</td>
<td>0.640*</td>
<td>0.627</td>
</tr>
</tbody>
</table>

Adenylate nucleotides, hypoxanthine (Hx) and inosine (Ino) made up less than 20% of the measured nucleotide pool for sardines and redbait at landing (Table 4). Most of the change in K factor during storage and thawing experiments was due to reduction in IMP as shown below (Fig 6).

Table 4 Mean percentage (+ SE) nucleotides at the jetty (sardines, n = 52) or net and factory combined (redbait, n = 8)

<table>
<thead>
<tr>
<th></th>
<th>Sardines</th>
<th>SE</th>
<th>Redbait</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP</td>
<td>80.83</td>
<td>0.42</td>
<td>79.71</td>
<td>0.93</td>
</tr>
<tr>
<td>ATP</td>
<td>2.17</td>
<td>0.15</td>
<td>2.14</td>
<td>0.68</td>
</tr>
<tr>
<td>ADP</td>
<td>4.12</td>
<td>0.17</td>
<td>4.26</td>
<td>0.18</td>
</tr>
<tr>
<td>AMP</td>
<td>0.89</td>
<td>0.12</td>
<td>0.55</td>
<td>0.03</td>
</tr>
<tr>
<td>Hx</td>
<td>5.27</td>
<td>0.30</td>
<td>4.61</td>
<td>0.13</td>
</tr>
<tr>
<td>Ino</td>
<td>6.72</td>
<td>0.25</td>
<td>8.74</td>
<td>0.61</td>
</tr>
</tbody>
</table>

IMP remained stable during short-term storage (40 days) but declined by up to 30% over longer periods, depending on species (Fig 6, Table 5). In each case Hx and Ino rose as IMP declined with redbait showing the greatest change.
**Fig 6 Sardines and redbait: short and long term storage effects on nucleotides**

Data were not available for short-term redbait storage. IMP – Inosine monophosphate, Hx – Hypoxanthine, Ino – Inosine. Fitted lines are significant regressions ($P<0.01$).
Table 5 Percent change in IMP, Hx and Ino from baitfish under long term storage. Refer Fig 6

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Storage period (d)</th>
<th>IMP</th>
<th>Hx</th>
<th>Ino</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sardines</td>
<td>Block</td>
<td>170</td>
<td>-17</td>
<td>25</td>
<td>56</td>
</tr>
<tr>
<td>“</td>
<td>IQF</td>
<td>170</td>
<td>-26</td>
<td>23</td>
<td>33</td>
</tr>
<tr>
<td>Redbait</td>
<td>Block</td>
<td>186</td>
<td>-29</td>
<td>61</td>
<td>68</td>
</tr>
</tbody>
</table>
iii. Histamine

Histamine levels were variable but very low during sardine and redbait frozen storage trials. Levels ranged from 0.5 to 6ppm, well below the critical level for human consumption (50ppm, USFDA, 2001).

C. Fresh refrigerated sardines

i. Vitamins

Marked reductions in vitamins C and E (Figs 7 & 8, Table 6) were observed when fresh sardines were kept for four days at 4°C. Vitamin E declined least during immersion in freshwater (Fig 8, Table 6).

Fig 7 Sardines: change in vitamin C during refrigerated storage of fresh fish at 4°C

- Saltwater
- Freshwater
- Air

<table>
<thead>
<tr>
<th>Day</th>
<th>Vitamin C mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

- Saltwater
- Freshwater
- Air

<table>
<thead>
<tr>
<th>Day</th>
<th>Vitamin C mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
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<tr>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

- Saltwater
- Freshwater
- Air

<table>
<thead>
<tr>
<th>Day</th>
<th>Vitamin C mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>25</td>
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<tr>
<td>1</td>
<td>20</td>
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<td>2</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

35
Fig 8 Sardines: change in vitamin E during refrigerated storage of fresh fish at 4°C
Table 6 Vitamin C and E: % losses in fresh sardines during 4 days storage at 4°C

<table>
<thead>
<tr>
<th></th>
<th>Experiment</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>a) Vitamin C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltwater</td>
<td>53</td>
<td>58</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Freshwater</td>
<td></td>
<td>63</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>24</td>
<td>69</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td><strong>b) Vitamin E</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltwater</td>
<td>33</td>
<td>62</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Freshwater</td>
<td></td>
<td>35</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>39</td>
<td>75</td>
<td>59</td>
<td></td>
</tr>
</tbody>
</table>
ii. **TBARS and Nucleotides**

TBARS increased over four days (Fig 9), as did K factor (Fig 10) although the former showed some variability in the magnitude of the response. The greatest consistent change in TBARS occurred in air and the least in freshwater.

**Fig 9 Sardines: change in TBARS during refrigerated storage of fresh fish at 4°C**

![Graph showing change in TBARS during refrigerated storage of fresh fish at 4°C in saltwater, freshwater, and air.](image)
Fig 10 Sardines: change in K Factor during refrigerated storage of fresh fish at 4°C

- Saltwater
- Freshwater
- Air

a) Refrigerated Fresh I
b) Refrigerated Fresh II
c) Refrigerated Fresh III
There was a clear decline in IMP (35-61%) over this period (Fig 11) and a rise in inosine. Although K Factor showed no treatment effects, the loss of IMP was slightly lower in air than seawater (i.e. 19% in Fig 11); this was repeated over the three experiments (Fig 10) with a difference in loss of IMP between 7 and 42%.

**Fig 11 Sardines: change in nucleotides (μmol/g) during refrigerated storage of fresh fish at 4°C; Experiment III**

Figures on the lines are % losses of IMP over the thawing period.
SW - Seawater, FW – Freshwater, IMP – Inosine monophosphate, Hx – Hypoxanthine, Ino - Inosine
There was variability in the detection of histamine build-up during refrigerated storage with two experiments showing clear increases and one showing very little change. Histamine levels varied greatly over four days’ refrigerated storage (Fig 12), reaching a maximum of 83ppm (Fig 12b), 33ppm over the critical level for human consumption (50ppm). The same experiment showed 42-50ppm histamine by Day 2.

**Fig 12 Sardines: change in histamine (ppm) during refrigerated storage of fresh fish at 4°C**

- **Saltwater**
- **Freshwater**
- **Air**

---

### a) Refrigerated Fresh I

![Graph showing histamine levels for Refrigerated Fresh I](graph.png)

### b) Refrigerated Fresh II

![Graph showing histamine levels for Refrigerated Fresh II](graph.png)

### c) Refrigerated Fresh III

![Graph showing histamine levels for Refrigerated Fresh III](graph.png)
D. Thawing Trials

i. Vitamins

Vitamins were significantly reduced during the thawing experiments. Vitamin C declined markedly, more so if fish were thawed in water either refrigerated or at room temperature (Fig 13, Table 7). Fish that were thawed, refrozen then re-thawed (TFT, Fig 13) showed similar patterns.

Fig 13 Sardines: change in vitamin C during thawing experiments at room temperature or 4°C (refrigerated)
TFT = fish that were thawed, re-frozen then re-thawed

![Graphs showing Vitamin C levels during different thawing trials](image-url)
Redbait vitamin C declined less than that for sardines under the same thawing treatments (Fig 14, Table 7), but the former species had generally lower vitamin C concentrations at the beginning of the thawing process (compare Figs 13 & 14). Fish that were thawed, refrozen then re-thawed showed similar patterns to those thawed once only.

Table 7 Vitamin C: % losses in sardines and red bait during 3-4 days thawing in air, saltwater or freshwater

Fish were thawed in a walk-in refrigerator (4°C) or at room temperature

<table>
<thead>
<tr>
<th></th>
<th>Refrig blocks</th>
<th>Room temp blocks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>A) Sardines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltwater</td>
<td>86</td>
<td>57</td>
</tr>
<tr>
<td>Freshwater</td>
<td>85</td>
<td>84</td>
</tr>
<tr>
<td>Air</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>B) Redbait</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltwater</td>
<td>74</td>
<td>43</td>
</tr>
<tr>
<td>Freshwater</td>
<td>68</td>
<td>41</td>
</tr>
<tr>
<td>Air</td>
<td>15</td>
<td>36</td>
</tr>
</tbody>
</table>
Fig 14 Redbait: change in vitamin C during thawing experiments at room temperature or 4°C (refrigerated)
TFT = fish that were thawed, re-frozen then re-thawed
Vitamin E was also significantly reduced during the thawing process (Figs 15 & 16). Sardines and redbait appeared to lose less vitamin E when thawed in freshwater and most if thawed in air (Table 8). Thawing, re-freezing then re-thawing blocks had no effect on these trends.

**Fig 15** Sardines: change in vitamin E during block thawing experiments at room temperature or 4°C (refrigerated)

TFT = fish that were thawed, re-frozen then re-thawed. Symbols without connecting lines are TFT for which samples were taken on Day 0 and Day 4

- **Saltwater**
- **Freshwater**
- **Air**

**Saltwater T/F/T**
**Freshwater T/F/T**
**Air T/F/T**

**Vitamin E (mg/kg)**

**Day**

(a) Room Temperature

(b) Refrigerated
Fig 16 Redbait: change in vitamin E during block thawing experiments at room temperature or 4°C (refrigerated)

TFT = fish that were thawed, re-frozen then re-thawed. Symbols without connecting lines are TFT for which samples were taken on Day 0 and Day 4

- Saltwater
- Freshwater
- Air

- Saltwater T/F/T
- Freshwater T/F/T
- Air T/F/T

---

**a) Room Temperature**

![Graph showing vitamin E levels over days for Room Temperature conditions.]

**b) Refrigerated I**

![Graph showing vitamin E levels over days for Refrigerated I conditions.]

**c) Refrigerated II**

![Graph showing vitamin E levels over days for Refrigerated II conditions.]

---

Day
Table 8 Vitamin E: % losses in sardines and redbait during 3-4 days thawing in air, saltwater or freshwater
Fish were thawed in a walk-in refrigerator (4°C) or at room temperature

<table>
<thead>
<tr>
<th></th>
<th>Room temp</th>
<th>Refrig 1</th>
<th>Refrig 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A) Sardines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltwater</td>
<td>53</td>
<td>55</td>
<td>-</td>
</tr>
<tr>
<td>Freshwater</td>
<td>24</td>
<td>23</td>
<td>-</td>
</tr>
<tr>
<td>Air</td>
<td>-</td>
<td>72</td>
<td>-</td>
</tr>
<tr>
<td><strong>B) Redbait</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saltwater</td>
<td>3</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Freshwater</td>
<td>-</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Air</td>
<td>25</td>
<td>61</td>
<td>70</td>
</tr>
</tbody>
</table>
ii. TBARS and Nucleotides

TBARS and K factor increased significantly for both sardines and redbait during the thawing trials (Figs 17-20). The thawing environment (i.e. SW, FW or Air) had no effect on K Factor although TBARS were greater in air treatments for both species. Fish that were thawed, refrozen then re-thawed showed similar patterns to those thawed once only.

Fig 17 Sardines: change in TBARS (mg/kg) during thawing experiments at room temperature or 4°C (refrigerated)
TFT = fish that were thawed re-frozen then re-thawed

---

**Diagram Description:**
- **a) Refrigerated Block Thaw I**
  - Saltwater
  - Freshwater
  - Air
  - Saltwater T/F/T
  - Freshwater T/F/T
  - Air T/F/T

- **b) Refrigerated Block Thaw II**
  - Similarly plotted data points as above.
Fig 18 Redbait: change in TBARS (mg/kg) during thawing experiments at room temperature or 4°C (refrigerated)

TFT = fish that were thawed re-frozen then re-thawed. Symbols without connecting lines are TFT for which samples were taken on Day 0 and Day 3.
Fig 19 Sardines: change in K factor (%) during thawing experiments at room temperature or 4°C (refrigerated)

TFT = fish that were thawed re-frozen then re-thawed. Symbols without connecting lines are TFT for which samples were taken on Day 0 and Day 3

- Temperature conditions:
  - Room Temperature
  - Refrigerated

- Water conditions:
  - Saltwater
  - Freshwater
  - Air

- Thawing phases:
  - Block Thaw
  - Block Thaw II

The graphs show the change in K factor (%) over time for different temperature and water conditions.
Fig 20 Redbait: change in K factor (%) during thawing experiments at room temperature or 4°C (refrigerated)

TFT = fish that were thawed re-frozen then re-thawed. Symbols without connecting lines are TFT for which samples were taken on Day 0 and Day 3.
IMP was significantly reduced during thawing for both species, particularly at room temperature (by 92 – 99%, Fig 21 & 22, Table 9). Further degradation of IMP presumably contributed to the observed increases in Hx and Ino although there is some quantity of IMP unaccounted for. There were no clear treatment effects.

**Fig 21** Sardines: change in nucleotides (μmol/g) during thawing experiments at room temperature or 4°C (refrigerated)

SW - Seawater, FW – Freshwater, IMP – Inosine monophosphate, Hx – Hypoxanthine, Ino – Inosine

![Graph showing changes in nucleotides during thawing experiments.](image-url)
Day 0 2 4 6 8 10 12 14

Nucleotide (μmol/g)

Day

c) Refrigerated Block Thaw II

Nucleotide (μmol/g)

Day

0 1 2 3 4

0 2 4 6 8 10 12

53
Fig 22 Redbait: change in nucleotides ($\mu$mol/g) during thawing experiments at room temperature or 4°C (refrigerated)

SW - Seawater, FW – Freshwater, IMP – Inosine monophosphate, Hx – Hypoxanthine, Ino – Inosine
Table 9 Percent loss of IMP from fish in short-term block-thawing experiments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Storage period (d)</th>
<th>% Loss by Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(d)</td>
<td>SW</td>
</tr>
<tr>
<td>Sardine</td>
<td>Room Temp</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Refrigerated I</td>
<td>4</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Refrigerated II</td>
<td>4</td>
<td>47</td>
</tr>
<tr>
<td>Redbait</td>
<td>Room Temp</td>
<td>3</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Refrigerated I</td>
<td>4</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Refrigerated II</td>
<td>4</td>
<td>54</td>
</tr>
</tbody>
</table>
iii. Changes in Histamine

Histamine levels were extremely variable for both species (Fig 23 & 24), particularly during room temperature thawing trials. In one case the level was almost ten times published critical levels for human consumption (50ppm) with 443ppm recorded in the sardine trial after thawing for 3 days at room temperature.

Fig 23 Sardines: change in histamine (ppm) during room temperature block thawing experiments

TFT = fish that were thawed re-frozen then re-thawed. Symbols without connecting lines are TFT for which samples were taken on Day 0 and Day 3

---

![Graph a) Room Temperature Block Thaw](attachment:graph_a.png)

![Graph b) Refrigerated Block Thaw](attachment:graph_b.png)
Fig 24 Redbait: change in histamine (ppm) during room temperature block thawing experiment.

TFT = fish that were thawed re-frozen then re-thawed. Symbols without connecting lines are TFT for which samples were taken on Day 0 and Day 3.
E. Ice Hours

Ice hours were highest for room temperature air thaw over 73 hours (Fig 25), that is, an 8 fold increase in the deterioration that might be expected had those fish been kept on ice for that period. There was a reduction for wet thawing under the same conditions possibly the result of the buffering effect of the water on diurnal temperature fluctuations. The thaw-freeze-rethaw treatments were lower again as these treatments were placed in a -12°C freezer for 24 hours in the middle of the trial. Refrigerated thawing provided the lowest ice hours. These data originate from loggers included with experiments.

Fig 25 Accumulated ice hours during thawing experiments at room temperature (Mean 18.56°C, Range 13-25°C) or in a commercial refrigerator (Mean 1.58°C, Range 1.0-3.0°C) over 73 hours

Refrigerator thawing trials were run for 96 hours. Ice hours calculated from these trials were scaled down by 73/96 (i.e. 0.760) to allow comparison with the 73 hour room temperature trials.
3. DISCUSSION

A. Vitamins

Vitamins are critical to the health and growth of SBT. Vitamin E is considered the most important natural antioxidant (Huss, 1995), blocking free radical chain reactions arising during the process of lipid peroxidation (Gouillou-Constans and Guillaume, 2001). These free radicals would otherwise contribute to the peroxidation of highly unsaturated fatty acids (HUFAs) which are very common in fish tissue (Corraze, 2001, Sargent et al., 2002) and generate intermediate toxic products such as malonaldehyde (Kubow, 1992), used as a measure of TBARS in this study. In this role lipid-soluble Vitamin E contributes to *in vivo* integrity of cell membranes and lipids in general and slows down the post-mortem development of rancidity (Gouillou-Constans and Guillaume, 2001) and tissue damage (Corraze, 2001). Vitamin C is water soluble and may spare vitamin E at the cell membrane surface (Sealey and Gatlin, 2002), that is, quench free radicals that would otherwise consume vitamin E. Sealey and Gatlin suggested that this could go both ways with each vitamin taking part in the quenching process. Regeneration of vitamin E by vitamin C at the membrane surface may also occur (Huss, 1995, Hamre et al., 1997, Sealey and Gatlin, 2002, Becker et al., 2004).

In either case these vitamins act to preserve the integrity of baitfish as a food source and contribute to the maintenance of health and growth of SBT through protection of lipids in food and their roles as vitamins once ingested by the fish (Halver, 2002). Conversely, the feeding of oxidised lipid, and associated free-radical-generated toxic compounds, to fish has been found to reduce growth, appetite and feed efficiency and to increase mortality (Sargent et al., 2002) in numerous species of fish including carp (Hata and Kaneda, 1980), channel catfish (Murai and Andrews, 1978), yellowtail (Park, 1978), rainbow trout (Cowey et al., 1984), Atlantic and coho salmon (Ketola, 1983) and African catfish (Baker and Davies, 1996).

The greatest changes in both nutritional and anti-nutritional factors occurred during thawing over 3 to 4 days. The quantity of vitamins remaining in the tissue was clearly inadequate to reduce the tissue-lipid oxidation rate if
baitfish were stored for long periods, or thawed for 2-4 days, as it rapidly fell to low levels, well below that included in pellets feed to SBT (Table 10a and b). The oxidation cascade is temperature dependant (Buchanan and Thomas, 2005), but still occurs at low temperatures (e.g. a -20°C commercial freezer) as shown in the present study.

Freezing method (i.e. IQF or blocks) influenced the degree of vitamin E loss. The higher initial vitamin E level for IQF sardines was possibly a seasonal effect although the trials began just 1 month apart (IQF: 13-14/05/05, Block 20/04/05). As the trial continued differences were probably related to freezing method. Individually frozen fish may have been more exposed to atmospheric oxygen than those frozen together in blocks, allowing greater oxidation pressure and therefore loss of vitamins.
Table 10a Published vitamin C and E requirements and Skretting’s pellet minimum vitamin inclusion levels for SBT (mg/kg dry pellet)

<table>
<thead>
<tr>
<th>Species</th>
<th>Vit C</th>
<th>Vit E</th>
<th>Reference:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bass (Hybrid striped)</td>
<td>22</td>
<td>80-100</td>
<td>Sealey and Gatlin, 1999</td>
</tr>
<tr>
<td>Carp</td>
<td>30-50</td>
<td>80-100</td>
<td>Halver, 2002</td>
</tr>
<tr>
<td>Catfish (Channel)</td>
<td>11-60</td>
<td>25-50</td>
<td>NRC, 1993</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>30</td>
<td>Halver, 2002</td>
</tr>
<tr>
<td>Flounder (Olive)</td>
<td>93</td>
<td>50-100</td>
<td>Wang et al., 2002</td>
</tr>
<tr>
<td>Salmon (Pacific)</td>
<td>50</td>
<td>40-50</td>
<td>NRC, 1993</td>
</tr>
<tr>
<td>Salmon (Atlantic)</td>
<td>50</td>
<td>35</td>
<td>NRC, 1993</td>
</tr>
<tr>
<td>Tilapia (Blue)</td>
<td>50</td>
<td>25</td>
<td>NRC, 1993</td>
</tr>
<tr>
<td>Tilapia (Nile)</td>
<td>42</td>
<td>50-100</td>
<td>Soliman et al., 1994</td>
</tr>
<tr>
<td>Trout (Rainbow)</td>
<td>40</td>
<td>25-100</td>
<td>NRC, 1993</td>
</tr>
<tr>
<td></td>
<td>100-150</td>
<td>30</td>
<td>Halver, 2002</td>
</tr>
<tr>
<td>Yellowtail</td>
<td>122</td>
<td>119</td>
<td>NRC, 1993</td>
</tr>
</tbody>
</table>

SBT pellet inclusion level | 60 | 100 |

Table 10b Baitfish wet weight equivalent to SBT pellet minimum vitamin inclusion levels (mg/kg wet wt)

<table>
<thead>
<tr>
<th>% Moisture</th>
<th>Vitamin</th>
<th>Vitamin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sardine</td>
<td>73.6</td>
<td>17.6</td>
</tr>
<tr>
<td>Redbait</td>
<td>69.1</td>
<td>20.6</td>
</tr>
</tbody>
</table>

TBARS levels were generally low for sardines, probably correlated with the low fat content (mean crude fat 2.26%, range 0.58 - 6.97%, n=274), as also suggested by Grigorakis et al (2004) for cultured sea bass on ice. Redbait had a higher mean fat content than sardines (mean crude fat 6.62%, range 2.44 – 11.59%, n=218) but surprisingly did not show higher TBARS during equivalent block thawing experiments. A higher initial TBARS level might be expected, given redbait used in these trials had generally spent time in frozen storage before arriving in Port Lincoln. Redbait are more robust fish than sardines; this may extend to the rate of physical breakdown, including the exposure of fats to peroxidation.

It is likely that TBARS were underestimated, at least in long-term storage. During biochemical analysis TBARS were equated to malonaldehyde, but the
decay of the long term storage curves suggest further breakdown to other products over the period. A similar result was reported by Hardy et al (1983) for TBARS levels during long term storage of a dry salmonid diet.

B. K factor and Nucleotides

As expected, K factor increased during storage and thawing trials as nucleotides (particularly IMP) declined and inosine and hypoxanthine increased. ATP, ADP and AMP were very low at the beginning of all experiments (≤4% of nucleotide pool). Similarly, Losada et al (2004) found K factor increasing to 80% for European sardines (Sardina pilchardus) stored for 22 days on flake ice; values were significantly lower on slurry ice presumably because of its greater cooling capacity (Pineiro et al., 2004). Kuley et al (2005) reported an increase in K factor of gutted sea bream stored on ice for 15 days and Ozogul et al (2004) reported a K factor of 80% for sardines (Sardina pilchardus) kept on ice for 9 days, a similar daily rate of increase to the present study if the 4 day level is extrapolated to 9 days.

Nucleotides, including IMP, have been implicated in diet palatability, fish feeding behaviour, growth stimulation, broodstock fortification, stress tolerance and as modulators of innate and adaptive immune responses (Li and Gatlin, 2006). IMP in particular has been implicated as a feeding stimulant in a number of aquaculture species including turbot (Scophthalmus maximus) (Mackie and Adron, 1978), amberjack (Seriola dumerilii), grunt (Parapristipoma trilineatum) and rabbitfish (Siganus fuscescens) (Ishida and Hidaka, 1987). Scombrids (i.e. mackerels and tunas) such as jack mackerel (Trachurus japonicus) and chub mackerel (Scomber japonicus, Ishida and Hidaka, 1987) and Pacific bluefin tuna (Thunnus orientalis, Kohbara et al., 2006) also show feeding responses to IMP. There are other well documented stimulants including several amino acids (Atema, et al., 1980, Olsen et al., 1986, Ishida and Hidaka, 1987, Kohbara et al., 2006) but amino acids were not measured in the present study. These studies indicate that many scombrids are not just visual predators, but rely upon other cues when foraging for prey beyond the line of sight, as suggested by Atema et al (1980). Although it is not known to what extent baitfish IMP affects appetite and/or contributes to palatability for southern bluefin tuna, it is possible that the
significant loss of this component from baitfish during storage or thawing could reduce the feeding response, and thus SBT growth rate.

C. Relationships between vitamin levels and nutritional quality

There were very strong negative correlations between rancidity (TBARS) and freshness (K factor) and antioxidant levels (vitamin C and E) (Table 11), supporting the frequently reported role of vitamins in maintenance of post-mortem flesh quality (Boggio et al., 1985).

Table 11 Correlation between vitamin levels and freshness (K factor) and rancidity (TBARS) indices during storage trials and thawing experiments

All relationships are negative and P < 0.004. * Saltwater, freshwater and air data pooled from thawing experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Fish</th>
<th>Freezing method</th>
<th>Period</th>
<th>Vitamin</th>
<th>R²</th>
<th>TBARS</th>
<th>K factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage</td>
<td>Sardines</td>
<td>IQF</td>
<td>6wk</td>
<td>E</td>
<td></td>
<td>0.648</td>
<td></td>
</tr>
<tr>
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<td>0.690</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td></td>
<td>0.928</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>E</td>
<td></td>
<td>0.762</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Block</td>
<td>6wk</td>
<td>E</td>
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<td>0.455</td>
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<td></td>
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<td>C</td>
<td></td>
<td>0.390</td>
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<td>E</td>
<td></td>
<td>0.455</td>
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<td></td>
<td>Redbait</td>
<td>Block</td>
<td>9wk</td>
<td>E</td>
<td></td>
<td>0.666</td>
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</tr>
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<td></td>
<td></td>
<td>Block</td>
<td>6mo</td>
<td>E</td>
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<td>C</td>
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</tr>
<tr>
<td></td>
<td>Thawing*</td>
<td>Sardines</td>
<td>Block</td>
<td>4d</td>
<td>C</td>
<td>0.874</td>
<td>0.817</td>
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<td></td>
<td></td>
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<td>0.728</td>
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</tr>
</tbody>
</table>

63
Pooled TBARS data (i.e. those from storage and thawing experiments) also showed significant correlations with decline in % vitamin E level (i.e. vitamin E at each sampling point as a percentage of initial level) in both sardine and redbait tissue (Fig 26a and c). Fifty-seven and 85% of the variation in the % vitamin E/TBARS data was explained by regressions for sardines and redbait respectively. In contrast, the strongest correlation with K factor was 48%, for sardines (Fig 26b), with the remaining relationships explored for either parameter or species (eg. vitamin E with K factor) either non-significant (P>0.05) or explaining 20% or less of variation in the data.

Correlations between TBARS and remaining vitamin E in the pooled data suggest good utility of TBARS as an indicator of this antioxidant in both species with 1-1.5mg/kg TBARS equating to 50% remaining vitamin E in each case (Figures 26a and c). In view of this result it is recommended that TBARS be included in as an analytical requirement for baitfish quality analysis in Port Lincoln. Analysis of TBARS is also methodologically simpler (spectrophotometry cf HPLC) and less time-consuming than that for K factor. Analysts should be mindful of the possibility of the further breakdown of TBARS to malonaldehyde, as discussed above.
Fig 26 Baitfish TBARS (mg/kg) and K Factor (%) vs % vitamins (mg/kg) remaining: storage and thawing data combined

a) Sardines: TBARS (mg/kg) vs % vitamin E remaining (VEr) (mg/kg)
   TBARS = 2.887 x 0.985 VEr, R² = 0.571, P<0.001

b) Sardines: K factor (%) vs % vitamin C remaining (VCr) (mg/kg):
   K Factor = 45.442 - 0.291 x VCr, R² = 0.480, P<0.001
c) Redbait: TBARS (mg/kg) vs vitamin E remaining (VE\textsubscript{r}) (mg/kg)

TBARS = 4.609 - (0.097 \times VE\textsubscript{r}) + (0.001 \times VE\textsuperscript{2} \textsubscript{r}); R\textsuperscript{2} = 0.851, P<0.001

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D. Histamine

Histamine was highly variable but did increase, particularly during room temperature block thaw and refrigerated storage of sardines and redbait. The critical value for human consumption (50 ppm, USFDA, 2001) was exceeded several times but there are no data on the critical levels for SBT. Studies on other fish species suggest some symptoms but provide little evidence of impacts on feeding or growth. Histamine-supplemented fishmeal was reported to cause gastric lesions in rainbow trout (\textit{Onchorhynchus mykiss}) by Watanabe (1987) but Watanabe's methods were included in a similar study by Fairgrieve \textit{et al} (1994) and the lesions did not appear, although the trout did develop distended stomachs. Fairgrieve did not find any effect of histamine (2000ppm) on feed intake or growth but acknowledged that the 16-week trial period may not have been sufficient for their development. Shiozaki \textit{et al} (2004), also working on rainbow trout, reported that histamine dose did not affect growth rate or feed consumption but did cause gastric abnormalities. Opstvedt \textit{et al} (2000) did not find any specific effect of biogenic amines
(including histamine) on production performance or the gastrointestinal tract in Atlantic salmon (*Salmo salar*) although growth was reduced during feeding of stale fishmeal. They suggested that reduced growth and feed intake was caused in part by appetite-depressive factors in the fishmeal and that biogenic amines could be useful indicators of fishmeal freshness.

**BENEFITS AND ADOPTION**

Information on the reduction in sardine and redbait quality with storage and thawing, and recommendations on baitfish treatment have been communicated to Industry throughout the project during day to day contact and at SBT Aquaculture Subprogram-Aquafin CRC meetings. Feedback from industry representatives has been very positive and indicates likely uptake of key findings. Key results and recommendations have been detailed in a poster circulated to industry members (Appendix 3).

**FURTHER DEVELOPMENT**

Results will be incorporated into the Formubait® database, contributing to improvements in tuna feed cost effectiveness and performance.

**PLANNED OUTCOMES**

The results of this study have been made available to ASBTIA in a variety of formats including this report, a summary poster, annual SBT Aquaculture Subprogram Industry Workshop Handbooks, industry newsletters (Tuna Briefs; [http://www.sardi.sa.gov.au/pages/aquafin/southern_bluefin_tuna_publications.htm?sectID=967&tempID=11#Newsletters](http://www.sardi.sa.gov.au/pages/aquafin/southern_bluefin_tuna_publications.htm?sectID=967&tempID=11#Newsletters)), face to face discussions and formal presentations during the project itself and at Aquafin CRC meetings and the 2007 Aquafin CRC Conference. The data are also available for incorporation into the Formubait® database and will be published in a peer-reviewed journal (paper in preparation). The authors will work with those managing the Formubait® database to facilitate incorporation of relevant data and it is anticipated that adoption of the recommendations detailed in this report will contribute to evaluation of baitfish as source of vitamins, leading to more reliable (price, availability and quality) sources of feeds for tuna and to
CONCLUSIONS
The objectives were as follows:

1. To survey the consequences of existing harvest and post-harvest handling practices on measures of nutritional quality of local baitfish for feeding tuna.

2. To determine the effects of different harvest and post-harvest handling practices on measures of nutritional quality of local baitfish for feeding tuna.

3. To make recommendations to industry (baitfish fishers and tuna farmers) regarding existing or modified harvest and post-harvest handling practices that maximise the nutritional quality of local baitfish for feeding tuna.

Objective 1 was addressed with 2 fleet surveys and short (4-6 weeks) and long term (3-6 months) storage trials run using commercial freezers. Objective 2 was addressed with a series of thawing trials. All trials used both Australian sardines (*Sardinops sagax*) and redbait (*Emmelichthys nitidus nitidus*).

Existing harvest and post-harvest practices can have a significant impact on the nutritional quality of baitfish fed to southern bluefin tuna in seacages. In laboratory experiments simulating some commercial practices vitamins and nucleotides decline to low levels, rancidity increases and fish freshness declines significantly, to the point where the baitfish becomes of questionable utility, particularly as a source of antioxidants.

Freezing method affected the rate of vitamin E loss. IQF sardines stored at -20°C lost vitamin E at a higher rate than those which were block frozen, possibly because individually frozen fish are more likely to be exposed to air than those in a solid block.
Baitfish, particularly sardines, should be thawed in seawater, not air, to minimise vitamin losses. Vitamin E losses were actually least in freshwater and greatest in air, whereas for vitamin C losses were greatest at room temperature, least in refrigerated air. The seawater recommendation is a compromise to minimise losses of both vitamins. Air-thawing, particularly at room temperature, accelerates oxidative processes and thus irreversible breakdown of antioxidants within fish tissue.

Block feeding at sea is recommended as rapid thawing (1-2 hours vs 1-2 days) may reduce vitamin losses in comparison with factory storage and thawing. In addition, fish should be kept under ice when transferred from the fishing vessel’s refrigerated seawater tanks to the factory, and storage time limited.

Correlations between TBARS and remaining vitamin E suggest the utility of TBARS as an indicator of this antioxidant in both species, with 1-1.5mg/kg TBARS equating to 50% remaining vitamin E in each case. In view of this result it is recommended that TBARS be included in as an analytical requirement for baitfish quality analysis in Port Lincoln. Analysis of TBARS is also methodologically simpler and less time-consuming than that for K factor. Analysts should be mindful of the possibility of further breakdown of TBARS to malonaldehyde, as discussed above.

Objective 3 was addressed in consultation with the ASBTIA. It was decided that the most effective means of industry extension would be the production of a laminated summary of the key results for distribution to sardine fishers, processors and tuna farm operators. This was carried out. Key results were also included in industry newsletters (Tuna Briefs; http://www.sardi.sa.gov.au/pages/aquafin/southern_bluefin_tuna_publications.htm?sectID=967&tempID=11#Newsletters) and discussed with industry at SBT Aquaculture Subprogram-Aquafin CRC meetings at Port Lincoln. Results were also presented at the Aquafin CRC conference in the Barossa Valley, May 2007.
REFERENCES


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Sealey, W. and Gatlin, D., 2002. Dietary vitamin C and vitamin E interact to influence growth and tissue composition of juvenile hybrid striped bass (Morone chrysops x M. saxatilis) but have limited effects on immune responses. The Journal of Nutrition 132, 748-755.


APPENDICES

APPENDIX 1: INTELLECTUAL PROPERTY
There are no intellectual property issues arising out of this report

APPENDIX 2: STAFF
Andy Manning
Ben Zammit
Dr Richard Musgrove
Dr John Carragher
Dr Philip Thomas
Dr Jeff Buchanan
APPENDIX 3: LAMINATED POSTER DISTRIBUTED TO ASBTIA/BAITFISH INDUSTRY
NUTRITIONAL QUALITY OF BAITFISH

Effects of existing baitfish storage and thawing practices
- Fish nutritional quality declines
- Rancidity increases
- Vitamins and nucleotides decline

Recommendations to maintain nutritional quality:
- Use fresh fish within 48hrs if kept refrigerated, 24hrs if not
- Use at-sea frozen-block feeding
- Keep fish on ice from vessel’s RSW tank to factory
- Do not store frozen baitfish for longer than three months
- Use refrigerated seawater if thawing baitfish on land

Vitamin E losses were actually least in freshwater and greatest in air, whereas for vitamin C this was reversed in some trials. The seawater recommendation is a compromise to minimise losses of both vitamins.

Acknowledgements: TBOASA especially Ajka, Tony’s Tuna, Sekol, Stolt Seafarms, and AFE. Port Lincoln’s Sardine fleet; Sardine Temptations; Phil Thomas’s lab (LMSC), TAFI (U. Tas); Seafish Tasmania. This work formed part of a project of Aquafin CRC, and received funds from the Australian Government’s CRCs Program, the Fisheries R&D Corporation and other CRC Participants.

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