QUALITY CHANGES DURING ICED STORAGE OF WHOLE FRESHWATER PRAWNS
(Macrobrachium rosenbergii)

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The culturing and production of freshwater prawns (Macrobrachium rosenbergii) is rapidly emerging from the experimental stage into full-scale commercialization, and the species should soon take its place among other successful aquaculture enterprises such as salmon, trout, catfish and oysters. Harvesting statistics for freshwater prawns are sparse; however, in the state of Hawaii alone, annual production increased from 4,000 pounds in 1972 to 176,400 pounds through October 1979 (Lee, 1979). Production for 1979 was estimated to be in excess of 250,000 pounds but was much less than expected due to unusually cold weather which limited the supply of juveniles for stocking. The Hawaiian Prawn Producers Association estimates the 1981 production will be near 900,000 pounds. (Total U.S. landings for all marine shrimp species was approximately 336 million pounds for 1979 (USDC, 1979).)

Pioneering efforts in the breeding and culture of freshwater prawns began with the work of Shao Wen Ling in Penang, Malaysia, in 1959 and was continued by Takuji Fujimura in Hawaii (Meyers, 1974; Lee, 1979; Goodwin and Hanson, 1974). Since that time, most of the research effort has focused on hatchery and grow-out phases of production (Nip and Moy, 1979) and this phase of commercialization is now fairly well-defined. The next logical step is to concentrate on evaluating post-harvest handling methods and product quality parameters.

Although the results of a few studies have been reported, proper handling methods, maintenance of product quality and optimum storage conditions for harvested prawns have not been adequately addressed. Reports that are available reveal that research on product quality has been directed toward evaluating stability of the prawns during frozen storage (Nip and Moy, 1979; Miyajima and Cobb, 1977; Reddy, Nip and Tang, 1981; Hale and Waters (1981) ). Reports on the shelf life of iced freshwater prawns will keep on ice only 3 to 4 days before deterioration begins (Hanson and Goodwin, 1977; Miyajima and Cobb, 1977; Nip and Moy, 1979; Lee, 1979) but the actual data have not been reported in the literature. These workers suggest that the prawn hepatopancreas is extremely active enzymatically, and is chiefly responsible for the mushiness that develops during iced storage. Mushiness of the tail muscle is suggested as being the major factor contributing to the relatively short shelf life of whole iced prawns (Hanson and Goodwin, 1977; Goodwin and Hanson, 1974). Blanching at 65°C (150°F) for 15 seconds after chill-killing is reported to extend the shelf life to 4 to 6 days (Nip and Moy, 1979).

Iced storage is a convenient method of short-term preservation of prawns destined for market outlets in relatively close proximity to the
supply and the practice will probably continue as production increases. The shelf life of ice-stored whole prawns needs to be better defined and if it is only 3 to 4 days as reported, additional methods are needed to increase it substantially. The objectives of this study were to determine the shelf life of whole prawns held on ice (1) untreated, (2) blanched at 65°C (150°F) for 15 seconds and (3) dipped in a 50 ppm solution of chlorine for 1 minute. The latter two methods were selected because of previous reports of their effectiveness.

MATERIALS AND METHODS

Materials
Freshwater prawns were harvested from experimental ponds by South Carolina Marine Resources Research Institute (MRRI) personnel, spray-washed and chill-killed by immersion in ice water. They were transported to the laboratory in ice water tanks. Experimental samples were removed, mixed with flaked ice and refrigerated overnight. The prawns were processed approximately 18 hours after harvest. The average size of the whole prawns was 38 g or about 12 count per pound.

Thirty-nine pounds of whole prawns were removed from the ice and sub-divided into three equal groups of 13 lb. each. The first group was placed on ice and served as the control. The second group was immersed (in mass) in 40 gallons of water at 65°C (150°F) for 15 seconds, cooled for 1 minute in ice water and placed on ice. The third group was immersed in a 50 ppm chlorine solution (sodium hypochlorite) for 1 minute, drained and placed on ice. The three groups were iced in plastic containers (with provisions for the ice-melt to drain off as formed) and placed in a cooler at 4°C (39°F). The prawns/ice ratio was maintained at about one-one during the 20-day storage period. Representative samples of each treatment were removed at regular intervals and evaluated for total volatile nitrogen, pH, total aerobic plate count and sensory values. Peeled, raw meats from at least three prawns, or portions thereof, were used as subsamples in the microbial and chemical analyses of all sample treatments.

Methods
Chemical Analyses --- Total volatile nitrogen (TVN) analyses were conducted using the modified Conway microdiffusion technique described by Obrink (1955) and further modified by Cobb et al. (1973). The results are reported as an average of three replicates. The pH was measured by direct contact of a combination pH electrode with the raw, macerated flesh.

Microbial Analyses --- The microbial analyses consisted of determining the total aerobic plate count (TAPC) following procedures outlined in FDA’s Bacteriological Analytical Manual for Foods (AOAC, 1976). Standard plate count agar was used as the plating medium and the plates were incubated at 22°C (72°F) for 5 days. TAPC is reported per gram of sample and the results are the average of three replicates.

Sensory Evaluations --- All prawn samples were peeled, deveined and cooked by boiling for 2 minutes in 2% saltwater. They were then rinsed with cold water and refrigerated for 2 hours before presentation.
to the taste panelists. Organoleptic evaluations were made by six panelists at zero, 4, 6, 8, 11, 14 and 18 days of iced storage. Evaluations of color (light to dark), odor, flavor (mild to strong), texture (soft to firm) and acceptability (reject to accept) were recorded by each panelist by placing a vertical slash mark on a 200 mm long open scale line.

RESULTS AND DISCUSSION

Chemical and Microbiological Evaluations

The results of analyses for TVN, TAPC and pH are shown in Table 1. The TVN values for all treatments remained relatively unchanged throughout storage, although some fluctuation did occur. Analysis of variance (ANOVA) showed that there were significant differences in TVN (P ≤ 0.05) among treatments at 14 and 18 days of storage. TVN values for the chlorine-dipped and blanched samples were significantly higher than the controls at days 14 and 18. No explanation for this unexpected occurrence is offered. The lack of an overall increase in TVN values during storage indicated repression or lack of a significant number of proteolytic microorganisms and/or the absence of inherent proteolytic enzymes.

The initial TVN values for all treatments were substantially higher than those reported for penaeids (Cobb et al., 1973) and those reported for fresh water prawns in an earlier study by Hale and Waters (1981). The initial values (27-29.5 mg N/100g), in fact, correspond to those observed in penaeids held on ice for 15-18 days and characterized as spoiled. Values reported in this study, however, agree with those reported by Miyajima and Cobb (1977). A re-examination of prawns harvested in February 1981 from culture tanks at the South Carolina MRRI produced TVN values of 23-24 mg N/100 g of sample. The difference in TVN values for penaeids and freshwater prawns may be attributed to differences in feed ingredients, season of harvest, and/or environmental grow-out conditions for freshwater prawns.

The pH of prawns for all treatments increased progressively from 7.20 initially to a high of 7.75 for the control at 20 days of storage. The relatively slight increase in pH during storage indicates minimal breakdown of proteins releasing ammonia and amines and is reflected in the values obtained for TVN. The initial pH values found in this study correspond to those reported for ice-stored penaeids (Flores and Crawford, 1973). Our reported values agree with those of Nip and Moy (1979) but are higher than those shown by Hale and Waters (1981). Values reported for ice-stored penaeids increased from 7.2 to about 8.2 after 20 days and from 7.5 to about 8.5 for pandalids after 8 days of storage. Bailey et al. (1956) suggested that a pH of 7.7 or below is indicative of prime quality shrimp; those having values from 7.7 to 7.95 are poor quality but acceptable, and those having a pH of 7.95 or above were characterized as spoiled.

The TAPC for all treatments increased steadily during storage. Statistical analysis (ANOVA) showed that significant differences exist among treatments only on days 14 and 18. The TAPC increased through
Table 1. Total volatile nitrogen (TVN), pH and total aerobic plate count (TAPC) of ice-stored freshwater prawns.

<table>
<thead>
<tr>
<th>STORAGE PERIOD (DAYS)</th>
<th>TREATMENT</th>
<th>TVN (mg N/100g)</th>
<th>pH</th>
<th>TAPC (Log No./g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>26.76</td>
<td>7.20</td>
<td>4.79</td>
</tr>
<tr>
<td></td>
<td>Cl₂ dipped</td>
<td>29.44</td>
<td>----</td>
<td>5.03</td>
</tr>
<tr>
<td></td>
<td>Blanchéd</td>
<td>26.78</td>
<td>----</td>
<td>4.90</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>----</td>
<td>7.20</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Cl₂ dipped</td>
<td>----</td>
<td>7.20</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Blanchéd</td>
<td>----</td>
<td>7.25</td>
<td>----</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>28.95</td>
<td>----</td>
<td>5.38</td>
</tr>
<tr>
<td></td>
<td>Cl₂ dipped</td>
<td>26.88</td>
<td>----</td>
<td>4.67</td>
</tr>
<tr>
<td></td>
<td>Blanchéd</td>
<td>29.11</td>
<td>----</td>
<td>4.75</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>30.74</td>
<td>7.30</td>
<td>4.96</td>
</tr>
<tr>
<td></td>
<td>Cl₂ dipped</td>
<td>30.47</td>
<td>7.25</td>
<td>5.57</td>
</tr>
<tr>
<td></td>
<td>Blanchéd</td>
<td>28.51</td>
<td>7.30</td>
<td>4.28</td>
</tr>
<tr>
<td>11</td>
<td>Control</td>
<td>26.58</td>
<td>----</td>
<td>5.81</td>
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<tr>
<td></td>
<td>Cl₂ dipped</td>
<td>26.01</td>
<td>----</td>
<td>5.30</td>
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<tr>
<td></td>
<td>Blanchéd</td>
<td>27.14</td>
<td>----</td>
<td>5.83</td>
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<tr>
<td>14</td>
<td>Control</td>
<td>22.98</td>
<td>7.50</td>
<td>7.45</td>
</tr>
<tr>
<td></td>
<td>Cl₂ dipped</td>
<td>28.96</td>
<td>7.40</td>
<td>6.49</td>
</tr>
<tr>
<td></td>
<td>Blanchéd</td>
<td>29.79</td>
<td>7.45</td>
<td>6.57</td>
</tr>
<tr>
<td>18</td>
<td>Control</td>
<td>21.51</td>
<td>7.65</td>
<td>8.08</td>
</tr>
<tr>
<td></td>
<td>Cl₂ dipped</td>
<td>27.92</td>
<td>7.50</td>
<td>7.68</td>
</tr>
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<td></td>
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<td>26.04</td>
<td>7.60</td>
<td>7.32</td>
</tr>
<tr>
<td>20</td>
<td>Control</td>
<td>29.74</td>
<td>7.75</td>
<td>8.16</td>
</tr>
<tr>
<td></td>
<td>Cl₂ dipped</td>
<td>29.22</td>
<td>----</td>
<td>8.18</td>
</tr>
<tr>
<td></td>
<td>Blanchéd</td>
<td>----</td>
<td>----</td>
<td>8.02</td>
</tr>
</tbody>
</table>

about 3 log cycles (log 5 to 10g 8) with the control leading slightly after 11 days of storage. Flores and Crawford (1973) showed that the TAPC for ice-stored penaeids increased 2.5 logs after 10 days of storage and increased correspondingly with the pH. Alvarez and Koburger (1979) and Koburger et al. (1973), reporting on the iced storage of penaeids, showed an increase in TAPC of 2.5 logs after 10 days and 3 logs after 14 days of storage, respectively. Bieier et al. (1972) showed that the TAPC for rock shrimp (Sicyonia brevoirostra) increased about 2.5 logs after 14 days of ice storage.

Sensory evaluations of each sample at each storage time were quantified on the 200mm open scales. Analysis of variance was performed on the numerical data to determine significant differences among treatments. The average color scores of the six panelists for each treatment and time are exhibited in Figure 1. There was a general upward trend in color (darker) through 18 days of iced storage. The results indicated that the
Figure 1. Average color intensity at each storage period.
blanched sample was significantly darker than the control and chlorine dipped samples at 6 days but no significant difference was found at other time periods.

The average odor scores are shown in Figure 2. There was a steady increase in odor intensity of the cooked samples with no significant differences between treatments. Average flavor scores appear in Figure 3. There was a general increase in flavor intensity, accelerating after 14 days of storage. The treatments were significantly different (P ≤ 0.05) at day 6 only.

The average texture (firmness) ratings are shown in Figure 4. The results were variable and showed no real change in texture after 18 days but all samples were softer at 4 days than at zero, 6 or 8 days, and the blanched sample was softer than the others at 8 days. Mechanical shear values were determined for zero and 4-day iced samples (control) only as part of another study. The average values as measured with the Kramer shear press on 100 g samples were 372 lb force at zero time and 225 lb force at 4 days, a 40% decrease in force.

Average acceptability ratings of the panelists are shown in Figure 5. Acceptability was rated on personal preference considering all rating factors. There was a general downward trend in acceptability over the 18-day storage period, but the blanched and chlorine dipped samples recovered after a sharp decline over the first 4 days and then resumed a more gradual decline after 8 days. This is probably related to the perceived mushy texture of the experimental samples at 4 days followed by firmer texture ratings. Both experimental samples were significantly lower in acceptability than the iced control at 4 days (P ≤ 0.01), and the blanched sample was significantly less acceptable than the control and chlorine dipped samples (P ≤ 0.05) at 8 days.

CONCLUSIONS

In general, treatment of freshwater prawns with a 1-minute chlorine dip (50 ppm) or with a 15-second blanch at 65°C (150°F) did not substantially increase the iced storage life. Although chlorine and blanching treatments slightly delayed an increase in pH beyond 8 days of storage, the TVN values were not appreciably different from the control. Chlorine-dip and blanching treatments significantly inhibited the TAPC at 14 and 18 days as compared to the control. The relatively unchanged TVN values and minimal increase in pH indicated little proteolysis during storage. Apparently the proteolytic enzymes (except perhaps the collagenases), either inherent or microbially produced, remain inactive or react very slowly at ice storage temperatures. Although the TAPC increased at a constant rate, the population may not have included proteolytic microorganisms. Slight spoilage odors of the raw prawns were noted at 14 days of storage as reported by an informal panel of three technologists and were evident among all treatments. Mushiness of the raw tail muscle was observed on the 11th day for all treatments.
Figure 2. Average odor intensity at each storage period.
Figure 3. Average flavor intensity at each storage period.
Figure 4. Average texture at each storage period.
Figure 5. Average acceptability at each storage period.
REFERENCES


ACKNOWLEDGMENTS

The authors express gratitude to personnel of the South Carolina MMRI for supplying the freshwater prawns and Lysander Ng for statistical analysis of the experimental data.
DEVELOPMENT OF AN ENZYME AFFINITY ASSAY FOR SEAFOOD PRODUCTS

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Various methods have been developed to ascertain the quality of fish and fishery products. Organoleptic examination by trained inspectors is the most common method to evaluate seafood quality. Although this method is rapid and inexpensive, it is only semi-quantitative, but even trained inspectors often disagree as to what is unacceptable. In addition to being subjective, such tests rely upon the taste and odor of compounds that may be unrelated to the materials that are actually toxic, since toxic compounds in many cases have no taste or odor of their own. Thus, more quantitative, objective methods of evaluation are sought after by both the fishing industry and the consumer.

As early as 1937 Beatty and Gibbons (1) developed a technique to quantitate volatile amines, and demonstrated that the concentration of these amines could be correlated to decomposition in seafood. Bailey et al. (2) indicate that fish spoilage is derived from metabolic changes in tissue (autolytic changes), autooxidative processes, and bacterial action. Most investigators believe that bacterial enzymes are the major cause of spoilage.

Histamine, derived from the bacterial decarboxylation of the amino acid histidine has been implicated in the type of food poisoning known as scrombroid poisoning. Scrombroid poisoning is characterized by symptoms resembling those of histamine toxicity: flushing, palpitation, headache, and/or gastrointestinal upset. Although consumption of as much as 20 mg of pure histamine failed to produce these symptoms, its presence has been noted in spoiled fish and as a result an amount in excess of 50 mg percent is considered unsafe in food products (3).

A variety of chemical, biological and isotopic methods have been used to assay histamine. The lack of useful light absorption or emission properties requires that chemical analysis be based upon the properties of derivatives. Pure histamine in solution may be quantitated by various chemical methods, but the assay of biological samples usually requires purification before any chemical determination can be satisfactorily applied. The quantitative determination of histamine by coupling to a diazotized aromatic amine was first reported by Weiss and Ssobolew in 1914 (4). Koessler and Hanke (5) were first to use this reaction preceded by purification for the quantitative determination of histamine and imidazoles in general.

Diazoo coupling methods have two major obstacles to overcome: the instability of the colored product and the nonspecificity of the reaction (6). The achievement of adequate specificity is usually achieved by prior extraction of histamine either by chemical or chromographic
methods. The colored product can be partially stabilized with an adequate buffer followed by extraction of the product into methyl isobutyl ketone; however, the light absorption decreases by 7% per hour (7). This has been the accepted A.O.A.C. method until the development of the more specific phthalaldehyde procedure (8,9).

In 1959, Shore, Burkhalter and Cohn (10) observed that ortho-phthalaldehyde would condense with histamine under alkaline conditions. When the product is activated at 350 nm at low pH, it fluoresces at 450 nm with an intensity proportional to the histamine concentration. The reaction is of sufficient specificity that purification of the histamine derivative is not usually necessary—an advantage over other chemical methods. As with the diazo method, the stability of the derivative has been questioned.

During the past 10 to 15 years, immunoassays have gained wide acceptance as the method of choice for the routine analysis of a wide variety of substances because of their specificity, sensitivity, and relative ease. Such assays include radioimmunoassay (RIA), immuno-fluorescence and more recently enzyme-linked immunosorbent assay (ELISA) (11,12). In each case the most essential part of the assay is the availability of an antibody that is capable of specifically binding the toxin.

Although there are many modifications, ELISA techniques usually involve competition between the toxin in the sample and a known amount of toxin-enzyme conjugate for antibody binding sites. As a result of this competition, the amount of conjugate that binds to the antibody is inversely proportional to the amount of toxin in the sample. After separation of bound and unbound conjugate, the enzymatic activity of the bound conjugate is measured colorimetrically.

Antibody to histamine has been reported by various authors (13,14, 15,16). However, other investigators have not succeeded in obtaining useful quantities; in some cases injection of antigenic compounds may result in increased production of amine oxidases in the test animals. Both monoamine and diamine oxidase are physiologically capable of exerting a protective role against histamine (17,18).

For this reason, a substitute for histamine antibody was sought. Various proteins which are known to be capable of binding histamine are shown in Table 1. Of these, hog kidney diamine oxidase (DAO) was chosen because of its relative specificity and availability.

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Not yet isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1 and H2 cell receptors</td>
<td>Not yet isolated</td>
</tr>
<tr>
<td>Enzymes</td>
<td></td>
</tr>
<tr>
<td>N-methyl transferase (NMT)</td>
<td>Highly specific; not commercially available</td>
</tr>
<tr>
<td>monoamine oxidase (MAO)</td>
<td>Not specific; commercially available; unstable</td>
</tr>
<tr>
<td>diamine oxidase (DAO)</td>
<td>Relatively specific; commercially available; stable</td>
</tr>
</tbody>
</table>
DAO catalyzes the reaction shown in Figure 1. Crabbe and Bardsly (19) have shown that this enzyme reacts by a Ping Pong Bi-Ter mechanism with initial binding to the diamine. The deamination reaction, however, is quite slow compared to the initial binding; it requires four milligrams of commercial DAO to deaminate one micromole of histamine in one hour. Thus a negligible amount of histamine is deaminated during the three-minute duration of the assay. Of course, DAO will bind but not react with histamine conjugates in which the histamine moiety is converted to an amide or a secondary amine.

When the rate of binding by an enzyme is large compared to the rate of catalysis, the dissociation constant $k_{-1}/k_{1}$ is approximately equal to $k_m$. The $k_m$ value for DAO is $6.5 \times 10^{-5}$ at pH 7.4 (20). This can be compared to a dissociation constant of $10^{-5}$ for most hapten-antibody reactions (21). In other words, in the event that antibody to histamine should be obtained, it most likely would not bind better than DAO.

**METHODS AND MATERIALS**

**ELISA type histamine assay employing DAO Reagents**

1. **Histamine binding tubes.** Kimble 12 x 75 mm polystyrene culture tubes were incubated with 5 mg DAO (Sigma) in 2 ml distilled/deionized water at room temperature for varying periods of time. The contents were then poured off and the tubes incubated an additional 15 minutes with 5 mg/2 ml bovine serum albumin to reduce non-specific binding. The tubes were then rinsed five times to remove unattached protein.

2. **Histamine-peroxidase conjugate.** Histamine dihydrochloride (100 mg) and horseradish peroxidase (Sigma, 100 mg) were dissolved in 1 ml water, then reacted for 30 min. with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide HCl (600 mg). After dialyzing 24 hours, the product was lyophilized, and 0.05 mg/ml was dissolved in 0.01 M pH 6.5 phosphate buffer.

3. **Dye solution.** Ortho-dianisidine (0.3 mg/ml) was dissolved in 0.01 M pH 4.0 citrate buffer. Hydrogen peroxide (20 microliter/ml 3%) was added to the dye solution shortly before use.

4. **Stopping solution.** Six molar sulfuric acid was used to stop all enzymatic reactions.

**Assay procedure**

One ml of sample solution containing histamine was mixed with one ml histamine-peroxidase conjugate. This mixture was incubated in a histamine binding tube for 3 min. The mixture was then poured off and the histamine binding tube rinsed 5 times with distilled water. Two ml of dye solution was added to the tube. After 2½ min., the reaction was stopped by adding 2 ml of the stopping solution. The absorbance was then measured at 480 nm and compared to a standard curve.

**Histamine-inhibition precipitation reaction reagents**

1. **Histamine-binding suspension.** Carboxymethylcellulose hydrazide (200 mg) was converted to CM-cellulose azide by the addition of 5 ml 2.5% HCl followed by 10 mg NaNO₂. The precipitate was washed in cold water and again in 0.05 M phosphate buffer at pH 8.7. The cellulose derivative was then reacted with 10 mg DAO dissolved
\[ \text{Substrate (Histamine)} \quad \text{Enzyme (DAO)} \quad \text{Enzyme-substrate complex} \]

\[
\text{Dissociation constant } = \frac{k_1}{k_1} = \text{pH 6.3 } 8.3 \times 10^{-5} \quad \text{pH 7.4 } 6.5 \times 10^{-5} \quad \text{pH 8.5 } 3.5 \times 10^{-5}
\]

FIGURE 1. Effect of pH on Binding Constants for Diamine Oxidase}
in 5 ml of the same phosphate buffer. After the reaction, the
conjugate was washed and lyophilized.

2. **Precipitating reagent.** Bovine serum albumin (100 mg) and hist-
amine dihydrochloride (100 mg) were dissolved in 1 ml water, then
reacted for 30 min. with 1-ethyl-3-(3-dimethylaminopropyl carbo-
diimide HCl (600mg). The product was dialized 24 hours and
lyophilized.

**Assay procedure**

1. Approximately 5 mg of the AO-cellulose conjugate were dissolved
in 3 ml water forming a colloidal suspension having a milky
appearance. To this suspension was added the sample containing
histamine.

2. One-tenth ml of a 1 mg/ml solution of the precipitating reagent
was added to the above mixture. In the absence of sample histo-
mine, the suspension had very noticeable clumping followed by
precipitation.

**RESULTS AND DISCUSSION**

Variable amounts of diamine oxidase or other protein can be
attached to polystyrene tubes by changing the surface area, pH,
and incubation time. Figure 2 shows the effect of incubation time
compared to the DAO binding activity of the tubes. Some of the
enzyme denatured or was destroyed by bacterial action after 200
hours. By regulating the number of binding sites on the tubes,
one can change the sensitivity of the reaction.

Histamine-protein conjugates have been prepared using a variety
of bifunctional reagents. These have included diazotized para-
aminoacetanilide (14), glutaraldehyde followed by sodium boro-
hydride (22), oxidation of protein-bound carbohydrate to vicinal
aldehydes (23), 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide
HCl (CDI) (24), and others (25). CDI forms amide bonds between
free carboxyl groups on the protein and a primary amine. This
method of conjugation has been found to be reliable and does not
prevent bound histamine from binding to DAO or destroy appreciable
peroxidase activity. CDI conjugates of bovine serum albumin have
been prepared containing up to 11% histamine, determined by the
incorporation of tritiated histamine.

Evaluation of the various parameters affecting this assay method
are presented separately in another paper in these proceedings. The
assay requires less than 10 minutes, needs only a simple spectro-
photometer, and has been found to be easy and reliable. The pre-
cipitation test is being developed as a qualitative assay for use in
the field where laboratory equipment is unavailable. The principle
by which histamine inhibits lattice formation and consequently
agglutination and precipitation is shown in Figure 3.
Figure 2

Effect of Time on Diamine Oxidase Binding to Polystyrene
Figure 3
Inhibition of Aggregation by Free Histamine
CONCLUSION

Substitution of diamine oxidase—an enzyme with a high association constant for histamine and a slow rate of catalysis—for histamine antibody has allowed the extension of an enzyme-linked immunosorbent assay for a low molecular weight compound for which antibody is not available. This new approach has been designated Enzyme Affinity Assay (EASY).

LITERATURE CITED


QUANTITATION OF HISTAMINE IN TUNA USING AN ENZYME AFFINITY ASSAY

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The Enzyme Affinity Assay is a new technique which has recently been
developed in our laboratory as a means of determining the histamine con-
centration in tuna. Histamine is accepted as the best indicator for
assessing the quality of tuna fish. The present Association of Official
Analytical Chemists (AOAC) method for determination of histamine is
sensitive, reproducible and specific but requires careful analytical
techniques. An analyst doing only histamine analyses can do only about
30 samples a day. As a result only a small amount of the raw or pro-
cessed tuna is subjected to a histamine analysis.

The Enzyme Affinity Assay is similar in principle to the Enzyme
Immunoassay (EIA) introduced by B.K. van Weeman and A.H.W.M. Schuurs
in 1971 except that the Enzyme Affinity Assay requires no antibody.

In the EIA an antibody is affixed to a solid support such as a
poly styrene tube. Free antigen in the sample is then allowed to compete
with an enzyme-antigen conjugate for the antibody binding sites on
the tube. The amount of enzyme bound to the tube following washing
to remove all non-bound antigen, either free or conjugated, is inversely
proportional to the amount of antigen in the sample. In the Enzyme Affinity
Assay the antibody is replaced by a specific binding protein, such as
an enzyme. Thus there is no requirement for the ligand to be antigenic
or haptenic.

The present application of this method utilizes the enzyme diamine
oxidase (DAO) which specifically binds histamine, an indicator of spoilage
in tuna. Histamine, conjugated to another enzyme such as horseradish
peroxidase, is mixed with the histamine containing sample. This is then
introduced into a tube to which DAO has been adsorbed. Histamine in
the sample competes with the histamine-peroxidase conjugate for binding
sites on the DAO and, following washing to remove unbound peroxidase,
the amount of bound activity is an indication of the amount of histamine
in the original sample.

MATERIALS AND METHODS

Buffer Preparation
The primary buffer was 0.01 M sodium phosphate (pH 6.5) with
0.1 M NaCl (Buffer A). In some cases this same buffer at pH 7.3
was used (Buffer B).

Preparation of DAO coated tubes
Various concentrations of diamine oxidase (Sigma) were prepared
in deionized water, Buffer A or Buffer B. These solutions were then
introduced into polystyrene test tubes (Curtin-Matheson Scientific
Evergreen Scientific) and incubated at room temperature for varying periods of time. The tubes were then washed several times with water, Buffer A, Buffer B, Buffer A with 0.1% BSA (w/v) (Sigma) or Buffer A containing 0.5% (w/v) Tween 20. Some of the tubes were then incubated with other protein solutions as coating proteins and washed again several times. Tubes were stored at room temperature or frozen until used.

Preparation of Enzyme-Histamine Conjugates
Horseradish peroxidase (Sigma) or β-galactosidase was conjugated to Histamine (Sigma) using 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (Sigma) by the method of Goodfriend (1964).

Enzyme Assay
Horseradish peroxidase was assayed using o-dianisidine or 2-2'-azino-di- (3-ethyl-benzthiazolin-6'-sulfonate) (ABTS) (Boehringer-Mannheim) as substrate; o-dianisidine (15 mg) was dissolved in 50 ml of citric acid-NaOH buffer (pH 4.0); 0.05 ml of 3% H₂O₂ was then added; 0.5-2 0 ml of substrate was added to the assay tubes and incubated at room temperature for 5-10 minutes. Following the incubation 0.5-2 0 ml of 6 M H₂SO₄ was added to stop the reaction. The absorbance was then measured at 480 nm or 466 nm in a Bausch and Lomb Spectronic 2000 or in a Turner, Model 350, spectrophotometer. The ABTS procedure was that of Standefer and Saunders (1978).

β-galactosidase activity was determined using 0-nitrophenyl-β-D-galactopyranoside (Sigma) dissolved in Buffer B containing 0.01 mM MgCl₂ 920 mg/ml). Absorbance readings at 410 nm were made at intervals in the Turner spectrophotometer.

Enzyme Affinity Assay
For the enzyme affinity assay various dilutions of the enzyme-histamine conjugates, with added free histamine, were introduced into the DAO tubes, incubated for varying times at room temperature and then washed several times with one of the buffers. Enzyme substrate was then added and the enzyme assay performed as described above.

RESULTS AND DISCUSSION

Determination of method of choice for preparing DAO coated tubes
Variables which were considered to determine the best method for preparing the DAO coated polystyrene tubes are shown in Table 1. Twenty-four batches of tubes using various combinations of the listed parameters were prepared. The two most important variables were the concentration of DAO used and the coating protein used. The DAO concentration is critical to the sensitivity of the assay and will be considered in more detail below.
TABLE 1. Variables Affecting DAO Tube Preparation

<table>
<thead>
<tr>
<th>DAO Concentration (mg/ml)</th>
<th>Incubation Time</th>
<th>Diluent</th>
<th>Wash Solutions</th>
<th>Coating Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>several days</td>
<td>deionized water</td>
<td>deionized water</td>
<td>BSA</td>
</tr>
<tr>
<td>3</td>
<td>72 hours</td>
<td>Buffer A</td>
<td>Buffer A</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>1.5</td>
<td>36 hours</td>
<td>Buffer B</td>
<td>Buffer B</td>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>0.75</td>
<td>18 hours</td>
<td></td>
<td>Buffer A with 0.5%</td>
<td>Trypsin*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tween 20</td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>1 hour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The trypsin used was inactive.

Figures 1A and 1B shows comparative ratios for various coating proteins. These ratios show that the histamine-enzyme conjugate and/or the free histamine bind significantly to all coating proteins used, especially after 48 hr. incubation with the coating protein. Figure 1C shows the results of a typical assay using the trypsin coated tubes. Figure 1D shows the results obtained when five new tubes were washed five times with Buffer A containing 0.5% Tween 20 (Tween-Buffer A). The blank tube contained nothing but enzyme substrate. This indicates that after washing the tubes with Tween-Buffer A, there is almost no non-specific binding occurring.

The method of choice developed for preparation of the DAO tubes was to incubate with a DAO solution in Buffer A for varying periods of time, wash the tubes with Tween-Buffer A and either air dry them or freeze them until they were used.

Comparisons of different parameters of the enzyme affinity assay

Although tubes prepared using 0.1% BSA as a coating protein showed considerable non-specific binding, Figure 2 shows the results of assays run on these tubes. Tubes prepared with 0.75 mg/ml DAO solution were used for both assays.

Figure 3A shows the results of an assay using the ABTS substrate. This substrate has an advantage over the o-dianisidine in that the latter is carcinogenic. For this reason, the ABTS might be the substrate of choice. However, due to the longer incubation time required for ABTS, o-dianisidine was used for the majority of these assays. The DAO tubes for this assay were prepared with a DAO concentration of 10 μg/ml.

The assay shown in Figure 3B was performed in tubes prepared as part of the same batch as those used in the assay shown in 3A. This assay was performed using o-dianisidine as substrate. If the slopes of the two plots are compared, 3A has a slope approximately 1.5 times that of 3B. This might be another reason for selecting the ABTS over the o-dianisidine for use in this type of assay.
FIGURE 2. Effect of bovine serum albumin on non-specific binding.
FIGURE 3. Comparison of ABTS and o-dianisidine substrates.
A. Effect of incubation at 24 hours

B. Effect of incubation at 48 hours

24 hrs.  
Tubes incubated with:

- DAO-coating protein

- Coating Protein only

C. Enzyme affinity assay with trypsin coated tubes

D. Effects of detergent on non-specific binding

FIGURE 1. Evaluation of non-specific binding by coating proteins.
Figure 4 represents assays performed in an identical manner on tubes from two different manufacturers. The assay shown in A was performed in tubes from Curtin-Matheson and that in B in tubes from Evergreen. Both sets of tubes were incubated with 1 μg/ml DAO and treated identically throughout the procedure. The Curtin-Matheson tubes show a slightly greater slope than the Evergreen tubes. Of greater importance, however, is the fact that, in this particular assay, the Curtin-Matheson tubes show better linearity.

An illustration of the importance of balancing the conjugate concentration can be seen in Figure 5. Both assays were run on tubes prepared in the same batch. The only difference in the assays was that the conjugate used in B was more concentrated by a factor of about 1.4 than that used in assay A. It is readily apparent that, while the more concentrated conjugate gave higher absorbance values, reproducibility between tubes was much worse.

Another important point about the assay shown in Figure 5 A is that with a low DAO concentration (10 μg/ml) used to coat the tubes and the proper balance of conjugate it is possible to determine lower concentrations of histamine than has been seen in the previous assays.

**Evaluation of β-galactosidase-histamine conjugate**

The β-galactosidase-histamine conjugate prepared proved to be unacceptable for use in this assay. Using tubes with 1 μg/ml (the same ones used for the assay in Figure 5) the absorbance reading following a 1 hour incubation at 37°C was only 0.15.

**CONCLUSIONS**

The Enzyme affinity Assay appears to be an attractive method for the assay of histamine in tuna. The primary advantage of this method over the currently accepted AOAC method is that it is quick (the DAO binding step requires only 3 min. and the enzyme assay using peroxidase and 0-dionisidase as substrate, requires only 3 more min.) and does not require either highly trained personnel or the use of sophisticated equipment. It is even possible that the method can be adapted in the future to a semi-quantitative test which requires no instrumentation at all and can be done "in the field" by virtually anyone.

**REFERENCES**


FIGURE 4. Comparison of polystyrene tubes from different manufacturers.
FIGURE 5. Effect of conjugate concentration on assay.
REVIEW OF WASTE MANAGEMENT REGULATIONS AFFECTING THE SOUTHERN SEAFOOD INDUSTRIES

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University of Florida
Gainesville, Florida 32611

Waste management will continue to be a major problem facing the seafood processing industry in the 1980's (4). The current effluent guidelines for most seafood firms with direct discharge require waste treatment equivalent to 20 mesh screening and appropriate disposal of solids. EPA's concern for the level of compliance with these regulations has been demonstrated recently by letter inquiries mailed to various seafood firms throughout the southeast. The response has indicated compliance is lower than required. These inquiries have increased industry concern for waste management regulations.

Recent changes in permitting procedures will complicate efforts to comply with environmental regulations. Seafood firms must now complete a new consolidated NPDES permit. These permits require a more in-depth description of the processing operations. Changes in landfill requirements and increasing energy costs will limit solid waste treatment options.

Despite these current waste management problems, EPA is developing the second level of effluent limitation guidelines mandated for July 1, 1984. Preliminary results indicate these future guidelines may be 60 to 90 percent more restrictive. The controversial proposed technology is dissolved air flotation (DAF). EPA economic studies have indicated the DAF requirement could force closures of numerous southern seafood processing firms. Executive orders by the new Federal administration have established a presidential task force on regulatory relief and guidelines to reform the regulatory process. Various regulations, including DAF, will be reviewed with more attention to "cost-benefit" evaluations.

CURRENT SITUATION

The prevailing waste management regulations were mandated in the Federal Water Pollution Control Act Amendments of 1972 and 1977. The primary objective of these Clean Water Acts was to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters". The primary administrative authority to plan and enforce these Acts was the U.S. Environmental Protection Agency (EPA). Various State Environmental Regulatory Departments were established and adopted programs were patterned by the original acts.

The original regulatory plan was to specify a series of interim guidelines or goals which would lead to zero discharge into navigable waters by 1985. These guidelines are actual specified concentrations of various pollutants which could be permitted for discharge. The pollutants discharge from seafood processing plants are conventional pollutants, as opposed to toxic pollutants. Conventional pollutants include total
suspended solids (TSS), oil and grease (O&G), biological oxygen demand (BOD), fecal coliforms, and pH. The various guidelines of concentrations per industrial source are commonly referred to with acronyms (Table 1).

**TABLE 1. Schedule of interim guidelines for waste water regulations.**

<table>
<thead>
<tr>
<th>Source</th>
<th>Date for Compliance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Discharge</td>
<td>July 1, 1977</td>
</tr>
<tr>
<td></td>
<td>BPT</td>
</tr>
<tr>
<td>Municipal Discharge</td>
<td>July 1, 1984</td>
</tr>
<tr>
<td></td>
<td>BCT</td>
</tr>
<tr>
<td>New Source</td>
<td>1985</td>
</tr>
<tr>
<td></td>
<td>ZERO</td>
</tr>
<tr>
<td></td>
<td>Pretreatment Standards</td>
</tr>
<tr>
<td></td>
<td>New Source Performance Standards (NSPS)</td>
</tr>
</tbody>
</table>

BPT - Best Practical Technology.
BCT - Best Conventional Technology.

Municipal discharge or seafood effluents discharged into public owned treatment works (POTW) must comply with pretreatment standards which assure the wastes will not overload the facility, interfere with the municipal treatment process, or pass untreated. These standards are usually specified in a sewer use ordinance. Although common complaints are voiced against the limited carrying capacity and increasing costs of municipal waste treatment, most southern seafood plants discharging to municipal facilities have experienced no pretreatment regulations which have complicated the use of municipal facilities.

New source discharges are new seafood plants being constructed such that the installation may discharge pollutants. Effluent guidelines permitted for new sources are intended to be more stringent than for existing facilities with comparable operations. This form of regulation plans to prevent installation of obsolete technology.

Southern seafood plants with direct discharge into navigable waters\(^1\) must obtain NPDES permits (National Pollutant Discharged Elimination System). These permits can be issued by the regional EPA office in Atlanta, Georgia, and/or by the respective state environmental regulations office which has been approved for NPDES permitting. These permits specify the daily average and maximum concentrations of conventional pollutants which can be discharged from the plant. The sampling point for measuring concentrations is "at the end of the pipe," rather than after discharge into the receiving waters. Monitoring requirements are specified in the permit.

As noted in Table 1, the current EPA effluent guidelines for seafood plants with direct discharge are "best practical technology" (BPT). The various seafood categories are not permitted to discharge effluents containing pollutants in excess of the listed concentrations (Table 2).

\(^1\)Navigable waters does not imply navigability, but refers to all "waters of the United States".
TABLE 2. Current seafood processing effluent guidelines (BPT) in comparison with the suggested new guidelines (BCT) specified in EPA contract Report No. 68-01-3287.

<table>
<thead>
<tr>
<th>Seafood Categories</th>
<th>Existing Guidelines (BPT)</th>
<th>Suggested Guidelines (BCT)**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TSS*</td>
<td>OGC*</td>
</tr>
<tr>
<td>Shrimp, Southern</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-breaded</td>
<td>110**</td>
<td>36</td>
</tr>
<tr>
<td>(38)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Breaded</td>
<td>280</td>
<td>36</td>
</tr>
<tr>
<td>(93)</td>
<td>(12)</td>
<td></td>
</tr>
<tr>
<td>Blue Crabs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>2.2</td>
<td>.60</td>
</tr>
<tr>
<td>(.74)</td>
<td>(.20)</td>
<td></td>
</tr>
<tr>
<td>Mechanised</td>
<td>36</td>
<td>1.3</td>
</tr>
<tr>
<td>(12)</td>
<td>(4.2)</td>
<td></td>
</tr>
<tr>
<td>Oysters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand shucked</td>
<td>19</td>
<td>.77</td>
</tr>
<tr>
<td>(15)</td>
<td>(.70)</td>
<td></td>
</tr>
<tr>
<td>Steam &amp; Canned</td>
<td>270</td>
<td>2.3</td>
</tr>
<tr>
<td>(190)</td>
<td>(1.7)</td>
<td></td>
</tr>
<tr>
<td>Clams</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand shucked</td>
<td>59</td>
<td>.60</td>
</tr>
<tr>
<td>(18)</td>
<td>(.23)</td>
<td></td>
</tr>
<tr>
<td>Mechanized</td>
<td>90</td>
<td>4.2</td>
</tr>
<tr>
<td>(15)</td>
<td>(.97)</td>
<td></td>
</tr>
<tr>
<td>Menhaden†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>w/o solubles</td>
<td>2.6</td>
<td>3.2</td>
</tr>
<tr>
<td>(1.7)</td>
<td>(1.4)</td>
<td></td>
</tr>
<tr>
<td>with solubles</td>
<td>2.3</td>
<td>.80</td>
</tr>
<tr>
<td>(1.3)</td>
<td>(.63)</td>
<td></td>
</tr>
<tr>
<td>Catfish†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farm Raised</td>
<td>28</td>
<td>.60</td>
</tr>
<tr>
<td>(9.2)</td>
<td>(.20)</td>
<td></td>
</tr>
</tbody>
</table>

* TSS and OGC represent Total Suspended Solids and Oil and Grease, respectively.
** All pollutants are expressed in pounds of pollutant per 1000 pounds of raw product processed. The daily maximum limit is expressed in the top figures and the daily average limit is expressed in parenthesis in the lower figures.
*** The guidelines suggested in EPA Contract Report No. 68-01-3827 are expressed in pounds of pollutants per 1000 lbs of raw product. These figures were converted from the projected performance for selected End-of-Pipe treatment technologies listed in the report table 60 using model production figures from report tables 85 - 115.
† Menhaden and Catfish categories have guidelines for biological oxygen demand which are not noted in this table.
State agencies retain the authority to implement more stringent regulations, which, in most cases, assure compliance with state water quality standards. In situations which have no distinct seafood category, EPA can apply a "best engineering judgment" to set effluent guidelines in reasonable approximation of the established guidelines. For example, shrimp packaging houses in certain regions of the southeast cannot discharge settleable solids in excess of 15 ml/l as a daily average, and 25 ml/l as a daily maximum.

Thus a review of the current situation indicates southern seafood firms are attempting to comply with the existing regulations, but increasing energy costs and solid waste handling pose economic and technical problems which have not been solved. This situation must be considered when developing new regulations or the next interim of guidelines.

FUTURE SITUATION

In the near future seafood processing forms should anticipate three major changes in waste management:

1. Consolidated permitting
2. Pretreatment standards for municipal discharges
3. New effluent guidelines (BCT), the July 1, 1984 interim

Consolidated permitting became effective July 18, 1980. This was an attempt to improve the permitting process. Applicants for direct discharge permits must complete more detailed and complicated NPDES forms. The new forms (NPDES Form 2C) require:

1. Listing the latitude and longitude for each outfall to the nearest 15 seconds.

2. Preparing a line drawing showing the water flow through the facility from intake to discharge. The drawing must show water flow and balance through unit processes, production areas, sanitary flows, cooling water and stormwater runoff.

3. Listing of at least one analysis for biological oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), total suspended solids (TSS), ammonia, flow, temperatures, and pH. More specific analyses may be required for certain pollutants, i.e., fecal coliforms, oil and grease, residual chlorine, etc. All suspect toxic pollutants will be analyzed.

4. Signature certification by the vice president of a corporation or a general partner or proprietor of a partnership or sole proprietorship, respectively.

Industrial sources which depend on municipal waste treatment should anticipate increasing costs for waste treatment, more pretreatment standards and potential limits on municipal services. The 1980 census report indicates the most rapid population growth is in the southeastern region of the United States. Ten of the 20 most populated cities were located in Florida. Increasing populations reflect a demand for municipal waste treatment facilities and in areas along the southeastern coasts municipal facilities are not adequate to handle the existing demands. Future demands for municipal waste treatment could force more pretreatment
standards to control waste loads contributed from industrial sources. Likewise, the costs for construction and operation of municipal facilities may depend on more support through increasing cost for industrial users. This situation is further depressed by the Federal administration's recent budget proposal which recommends substantial reductions in EPA's construction grants for public-owned treatment works. Thus seafood processing firms which have avoided waste management problems by municipal discharge should anticipate future adjustments.

Direct discharges can anticipate the next step or interim guidelines for effluent limitations due July 1, 1984. To develop these guidelines, EPA has contracted the services of two consulting firms. One firm reported recommended waste treatment technology and effluent guidelines per various seafood categories (2). The second firm reported the economic impact assessment of the recommended technology in the first report (3). These reports were strongly contested by industry opposition which felt the recommended technologies were unnecessary, ineffective, and would impose severe economic burdens. The economic impact report is being revised for resubmission in late April, 1981.

The original technical report (2) recommended effluent guidelines that are 60 to 90 percent more restrictive than the existing BPT guidelines (Table 2). The recommended waste treatment technology is listed per seafood category in Table 3. The most controversial technology was dissolved air flotation (DAF). The DAF process used minute air bubbles to attach and float oil and grease and suspended matter from the carrying liquid. The separation process can be optimized by using flocculating agents like ferric chloride, alum, lime (pH 10-10.5), anionic polymers, and acid adjustments to lower pH.

**TABLE 3. Future waste treatment technology recommended in EPA Contract Report No. 68-01-3287.**

<table>
<thead>
<tr>
<th>Seafood Categories</th>
<th>Waste Treatment Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrimp, Southern--non-breaded</td>
<td>IP, S, DAF, SD</td>
</tr>
<tr>
<td>--breaded</td>
<td>IP, S, DAF, SD</td>
</tr>
<tr>
<td>Blue Crab--conventional</td>
<td>IP, GT, S</td>
</tr>
<tr>
<td>--mechanized</td>
<td>IP, GT, S, DAF</td>
</tr>
<tr>
<td>Oysters--hand shucked</td>
<td>IP, S</td>
</tr>
<tr>
<td>--steamed and canned</td>
<td>IP, S, GR, DAF, SD</td>
</tr>
<tr>
<td>Clams--hand shucked</td>
<td>IP, S</td>
</tr>
<tr>
<td>--mechanized</td>
<td>IP, S, GR, DAF, SD</td>
</tr>
<tr>
<td>Menhaden--without solubles</td>
<td>IP (extensive)</td>
</tr>
<tr>
<td>--with solubles</td>
<td>IP, B</td>
</tr>
<tr>
<td>Catfish--farm raised</td>
<td>IP, GT, S, AL</td>
</tr>
</tbody>
</table>

**Abbreviations:**
- IP - in plant modifications
- GT - grease traps
- GR - grit removal
- S - screening
- AL - aerated lagoons
- B - barging
- DAF - dissolved air flotation
- SD - DAF with sludge dewatering, only recommended for largest processing firms
There are numerous disadvantages to the dissolved air flotation treatment of seafood processing effluents:

1. Operational mode of a DAF unit requires lengthy start-up times (1-3 hours), continuous flow during operations, and lengthy shut-down and clean-up time (1-3 hours) (5). Most seafood processing operations are not a continuous process and vary daily and seasonally.

2. Trained, experienced labor is required to operate the DAF systems (6). This unique type of labor is limited and expensive. The seasonal schedule of production would be an unattractive feature for such highly trained labor.

3. High costs for DAF equipment, chemicals, power (energy), maintenance and operations, i.e.,

Estimated Costs* (figures from EPA Contract Report 68-01-3287)

<table>
<thead>
<tr>
<th>Plant Size</th>
<th>Days/Season</th>
<th>Capital</th>
<th>O&amp;M (annual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Breaded Shrimp</td>
<td>$1,000------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 tons/day</td>
<td>150</td>
<td>230</td>
<td>355</td>
</tr>
<tr>
<td>30 tons/day**</td>
<td>150</td>
<td>484</td>
<td>625</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant Size</th>
<th>Days/Season</th>
<th>Capital</th>
<th>O&amp;M (annual)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breaded Shrimp</td>
<td>$1,000------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 tons/day</td>
<td>150</td>
<td>205</td>
<td>293</td>
</tr>
<tr>
<td>12 tons/day</td>
<td>150</td>
<td>470</td>
<td>580</td>
</tr>
</tbody>
</table>

* Does not include cost for screening.
**Includes costs for sludge dewatering.

4. Disproportionate costs are higher on smaller size firms.

5. Land availability and costs are not considered in the original cost estimate figures. Coastal real estate is limited and expensive.

6. Sludge collected is a highly putrisensible scum (95% water) which must be disposed of in an environmentally sound manner.

7. Sludge odors can be a problem and will attract flies and rodents, which are unsanitary conditions in conflict with health and food processing regulations.

8. Sludge disposal is a major unanswered problem.
   a. Fewer landfills will accept sludge (95% water) and future environmental regulations could limit sludge disposal in landfills.
   b. The chemical additions during DAF treatment limit the use of sludge as a precursor in feeds or fertilizers.
   c. Likewise, ocean disposal, which is currently permitted for raw screened seafood solids, would be restricted for chemically treated seafood sludge.

9. Energy requirements for DAF treatment would increase energy use and processing.
The original economic impact assessment (3) prepared model economic profiles for each seafood category based on 1978 dates. Analysis of the model plants' impacts were primarily based on the determination of the model plants' net present value (NPV) both before and after expenditures for controls. The results indicated the southern shrimp industries would be the most severely impacted category (Table 4). The industry reviewers argue these results are very conservative, and if they were adjusted to reflect current economic conditions, the percent closures per category could exceed 50 percent. Likewise, the economic impacts on local communities and the balance in foreign trade have not been considered.

<table>
<thead>
<tr>
<th>Seafood Categories</th>
<th>Percent Plant Closures (%)</th>
<th>Annual Production Loss ($ million)</th>
<th>Employment Loss (# jobs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrimp, Southern--non-breaded</td>
<td>21</td>
<td>28.6</td>
<td>370</td>
</tr>
<tr>
<td>--breaded</td>
<td>32</td>
<td>36.3</td>
<td>1,590</td>
</tr>
<tr>
<td>Blue Crab--conventional</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>--mechanized</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Oysters--hand shucked</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>--mechanized</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Clams--hand shucked</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>--steamed and canned</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>Menhaden--without solubles</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>--with solubles</td>
<td>27</td>
<td>4.0</td>
<td>0</td>
</tr>
<tr>
<td>Catfish--farm raised</td>
<td>5</td>
<td>0.2</td>
<td>7</td>
</tr>
</tbody>
</table>

Currently, most seafood plants can comply with the BPT guidelines by employing some form of screening technology which preforms at a 20 mesh equivalent. This requirement is finer than the mesh in a typical screen door (14-15 mesh). Vibrating, rotary, and tangential screens have been used in conjunction with coarse bar screens, grit chambers and/or dry clean-up. The primary objective is to remove particulate matter or suspended solids, i.e., shells, scales, bones, etc. Recently, EPA has initiated letter inquiries to determine the level of compliance for screening effluents from southern seafood plants. The initial response has indicated compliance is lower than required, and failures to comply are more typical of the smaller firms (personal communication, EPA's Atlanta regional office, April, 1981). The inquiry is not complete and the utility of the results is questionable. These inquiries have increased industry concern for waste management regulations.

Limited compliance with screening requirements is due to the lack of benefit-incentives, costs of compliance, and no attractive options for disposal of solids. A common industry opinion is: "What comes from the sea can be returned with no harm, but should provide potential benefits." This theme has been recently reinforced by the EPA Section 74
Seafood Study which concluded "some coastal areas can assimilate or disperse large amounts of waste without serious effect." Thus industry reasons that, in some cases, compliance is an expense for the sake of treatment without benefits.

Unfortunately, the benefits of utilizing screened waste solids have not developed as anticipated. It is technologically feasible to use seafood solids to produce feeds, meals, fertilizers, chitosan, etc., but the logistics and economics of production are not reasonable. Unlike most food processing industries, the seafood industry has limited control on supply which fluctuates by season, month and week. Variations in raw materials complicate waste utilization and by-product marketing. If temporary storage is used to collect solids for by-product production, preservation is an additional expense. Temporary, unpreserved storage would violate good manufacturing practices regulated by other Federal and State agencies. Currently, by-products from seafood wastes compete unfavorably in a market with cheaper synthetics, and more consistent, higher quality products which can be produced in larger volumes. In the future, costs for producing some by-products will inevitably increase as cost for energy increases, i.e. dehydrated meals.

Most seafood firms depend on disposal for solid waste management. Public and private landfills are a popular disposal option. Associated problems are availability, disposal costs, transportation costs, and odor control. Ocean disposal is a viable alternative because untreated "fish wastes" do not require an EPA Ocean Dumping Permit (1). Despite this special exclusion, ocean dumping is not popular due to the difficulties of collection and transport of solids, so as not to attract flies and rodents and to suit variable weather conditions.

Thus, a review of the future situation indicates seafood waste management requirements could become more complicated and expensive. The consequences could be plant closures, decreased domestic production, and loss of jobs. The future regulations are presently being debated and the recent change in Federal Administration should shift the debate to favor more cost-benefit assessments to test the reasonableness of new regulations.

**PREDICTIONS AND RECOMMENDATIONS**

1. If production costs continue to increase, limited entry and import tariffs increase in popularity, and more stringent effluent regulations are adopted, the economic consequences could be devastating to the processing sector of the seafood industries in the Southeast.

2. Dissolved air flotation should be delayed or possibly omitted as a new seafood waste treatment technology. The tentative recommended proposal date for the new regulations (BCT) is July 1981. Changes in the federal administration and budget could delay the proposal. The original mandated date for compliance with the new regulations (BCT) is July 1, 1984. If the proposed regulations are finalized in 1982, industries will have less than two years to comply.
3. Existing regulations (BPT, screening) will be enforced. EPA compliance inquiries and potential elimination of new regulations will focus more regulatory attention on existing regulations. Due to anticipated budget cuts, EPA should focus more attention on toxic pollutants. Conventional pollutants should be ranked as a lower priority problem. Regardless of priorities, screening will most likely be enforced.

4. Industry and research efforts should be directed to determine economical and logistical methods for utilization and disposal of seafood solid wastes. New and old techniques should be investigated to consider the least cost option as well as potential benefits. Low energy production techniques to produce by-product substitutes for expensive, high energy products is advised, i.e. silages for feeds or fertilizer.

5. Seafood processing firms using municipal waste treatment should examine the potential for increasing cost for treatment and pretreatment standards. These points are often indicated in the sewer use ordinance. Alternative plans for waste treatment should be considered.

6. Industry and research efforts should examine methods for water conservation and recycling during seafood processing. Water recycling is a more difficult goal which must contend with regulatory approval to suit safe drinking water standards. Availability of fresh water is threatened in some coastal areas of the southeast. Eventually, permitting to restore water quantity could be used similar to permitting to preserve water quality.

REFERENCES


THE CRAWFISH INDUSTRY OF LOUISIANA: A REVIEW

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Cooperative Extension Service
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James E. Rutledge
Department of Food Science
Louisiana State University

Louisiana grows and processes 90% of the nation's crawfish. Last year in Louisiana, more than 60,000 acres were devoted to raising crawfish. The pond production coupled with the wild harvest yielded approximately 40 processing facilities in the state that cook, peel and package crawfish tail meat. Demand for live crawfish and crawfish meat usually exceeds supply. Growing markets outside the state have put additional demands upon this seafood. Historically, quality has been a major problem with this industry. Unlike many other food processing industries, the cooking, peeling and packaging of crawfish does not have definite recommended guidelines or specific government regulations for the production of a consistent product. Even though the typical crawfish processor will boil live crawfish and peel them by hand, the handling methods and procedures to do these steps may, and usually do, vary greatly from processor to processor. For example, one processor may cook his crawfish in boiling water for only 2 minutes, another may cook as long as 20 minutes. One processor may wash his crawfish several times before cooking, another may not wash his crawfish at all. Obviously, the omission of a processing step or a poorly conducted processing step may result in low quality.

Over the last decade, the typical crawfish peeler has changed from the small "ma and pa" operation to the more corporate patterned facility, often processing several thousand pounds of crawfish per day.

There are two distinct species of crawfish, the red swamp crawfish and the white river crawfish. Although a mixture of both species is trapped together, each species is adapted to the environment described by its name. The red swamp crawfish is by far the most common species harvested for human consumption, making up more than 60 percent of the catch.

The meat taken from the tail is currently the only economical source of meat from crawfish. On the average, crawfish will yield about 15% blanched meat from whole live crawfish. Small and medium sized crawfish will often yield more than 20% tail meat, but large mature crawfish yield only about 8% tail meat. As crawfish mature, the head and claws become proportionally larger than the tail, accounting for this significant difference in yield. Unlike shrimp and some other shellfish, crawfish are not graded according to size.

Crawfish production is not a year round processing business. In fact, fresh meat and live crawfish are available only from late November
until early June during good years. March, April and May are the months when crawfish are most plentiful and are of the best quality.

During normal years, the price of crawfish varies significantly throughout the season. For example, early in the season when production is from ponds—November, December, January and February—the prices are consistently high. However, in April the prices usually plunge when the wild crop begins to be harvested. When prices drop, processing increases drastically. This year, 1981, was an exceptional year. Wild crawfish production was down throughout the season. As a result prices remained high throughout the course of the season.

Crawfish are harvested by trapping. During the season, traps are emptied and baited daily. This procedure is highly labor intensive and adds considerable cost to the overall production of crawfish.

CRAWFISH PROCESSING

Crawfish processing may be shown in schematic using the following diagram of processing steps:

```
LIVE CRAWFISH ® WASH & INSPECT ® COOK ®

COOL ® PEEL ® WEIGH ®

PACKAGE ® ICE OR FREEZE ® CONSUMER
```
As noted in the diagram, the first processing steps are washing and inspecting the live product. A vat of water used in conjunction with water jets serves to remove mud and other debris. A conveyor belt lifts the crawfish from the water and inspectors remove dead crawfish and large pieces of extraneous matter such as bait. From the conveyor, live crawfish are placed into cooking baskets.

The cook consists of a rapid blanch, usually five minutes or less in boiling water. Over-cooked crawfish do not peel well because the meat often sticks to the shell.

Freshly cooked crawfish are usually placed on a table or in a holding bin to cool. Once cooled so that they can be safely handled, the crawfish are peeled by hand. Warm crawfish are generally easier to peel than cool or cold crawfish. For the most part, hand peeling is the norm, although mechanization is now possible and used by several plants in Louisiana. Crawfish peelers are paid according to the weight of meat peeled. A skillful worker can produce from eight to ten pounds of meat per hour. As the tail meat is removed it is placed into a colander. Periodically, the colander is emptied and the meat is weighed. Most bacterial problems associated with crawfish processing occur in the later stages of processing due to the degree of handling by workers and poor refrigeration practices.

Crawfish meat that is going to be sold fresh need only be packed into the desired package and immediately packed well on ice.

Crawfish meat that is to be frozen, however, requires more specialized handling. Upon receipt at the weighing station, the fat should be removed by washing in clean, cold water (about 40° F). If the fat is not completely removed, the meat may go rancid when frozen. Washing may be accomplished on a continuous basis using a conveyor belt and spray jets of water or on a batch basis using suitable tubs or vats. Prior to packaging and freezing, the meat should be dipped into a 0.5% citric acid solution. After packaging, the meat should be frozen at a temperature of 0° F or lower and maintained at that temperature.
ALLIGATOR MEAT: AN EVALUATION
OF A NEW SEAFOOD

Michael W. Moody, Paul Coreil and Jim Rutledge
Louisiana State University

Regulation changes within the last decade have permitted the taking of the American alligator, *Alligator mississippiensis*, in specified areas of South Louisiana. In 1972, it was determined by appropriate state and federal officials that sufficient numbers of alligators existed to provide a limited harvest in three southernwestern Louisiana parishes. In 1980, 12 parishes participated in the 30-day September season, taking approximately 20,000 alligators.

Initially, only skins could be introduced into commerce. In 1978, promulgation of revised regulations provided for the sale of alligator parts, including meat. Prior to this change, the meat was discarded or consumed by the trapper. With changed regulations that now permit sale of alligator parts in addition to the skin, there is growing interest in commercial marketing of the meat for human consumption. Such use will require modification in handling procedures to insure safe, wholesome meat products as well as baseline data on meat yields and nutritional characteristics. These topics were the objects of this study.

ALLIGATOR PROCESSING

Hunters normally capture alligators with baited hooks and kill them with a firearm at the site of capture. Alligator carcasses left from skinning have ordinarily been discarded as waste. New regulations by the State Food and Drug Administration require that alligator meat to be sold into intrastate commerce be prepared (including skinning) in a facility and by persons having a permit issued by the State Food and Drug Administration. Since alligators are classified as seafood, they need not be slaughtered within the confines of an approved facility, but still may be killed at the point of capture.

Twelve alligators of varying lengths and weights were captured by Louisiana Wildlife and Fisheries specialists with special handling equipment and transported live to the Louisiana State University meat processing facility in Baton Rouge. The alligators were slaughtered and processed in conformity with the Louisiana Sanitary Code. After killing, the lengths and weights were recorded. Each alligator was classified as small, medium, large or extra large. The animals were skinned, eviscerated and washed. The dressed carcasses were weighed. Other parts removed from the carcasses were also weighed separately. After chilling in a meat locker at 35°F for 12 hours, the carcasses were reweighed and butchered into four primary meat cuts. The cuts were from the tail, leg, torso and jaw muscles. The tail meat cut was obtained by cutting across the base of the tail just behind the hind legs. The leg meat cuts were obtained by severing the joints where the legs attach to the body. The jaw meat cut consisted of the jaw muscles. The remaining meat from the back and ribs was categorized as the torso meat cut. The only boneless cut was the jaw meat cut.
A proximal analysis (protein, fat, moisture and ash) of the various cuts of meats was determined. Analyses were run on only those portions of each cut that could be expected to be consumed. For example, excess fat was trimmed since alligator fat may impart a strong disagreeable flavor to the meat.

RESULTS AND CONCLUSION

Results from this study provide basic data to processors and other individuals considering marketing alligator meat in Louisiana. Tables 1 and 2 are basic yield data obtained in the study.

An interesting comparison was noted between the value of the skin and the value of the meat when both are considered on a weight basis. (The skin is normally sold according to length and not weight or area.) Last year this value was about $1 per inch. On a weight basis, the small alligator skins from the study were worth $19.82 per pound, the medium alligator skins were worth $9.81 per pound, the large alligator skins were worth $7.28 per pound and the extra large alligator skins were worth $3.47 per pound. As the size of the alligator increased, the value of the skin on a weight basis decreased. Consequently, as alligator size increased, the value of skin on a weight basis approached the value of the meat on a weight basis, assuming that the value of meat is constant, regardless of alligator size. Since the dressed weight is roughly 60 percent of the total live weight and the skin is roughly 15 percent, the value of the meat may be considerably higher than the skin on larger alligators.

The appearance of the meat cuts varied. For example, meat from the tail cut was white to light pink. Internal bands of hard, white fat— that appear circular in cross section—run lengthwise near the tail bone. Meat from the torso cut is similar to meat from the tail cut, except that it does not have the fat bands. The jaw meat is also white to light pink with no fat deposits. The leg meat, however, is darker color. Some small fat deposits were observed in the leg meat as well as a substantial amount of connective tissue and tendon.

Table 3 is the basic proximal analysis of the meat. The nutritional composition data compares favorably with other more traditional meats. While the fat content of the alligator meat ranges from 1.0 to 1.5 percent, the fat content of a choice grade beef rump roast is about 25.0 percent; for pork loin it is about 20.0 percent; for chicken (fryer, light meat without skin) it is 6.0 percent. The protein content of alligator meat ranges from 21.1 to 22.3 percent as compared to 17.0 percent of a choice grade rump roast; 13.0 percent for a pork loin; and 32.0 percent for chicken (fryers, light meat without skin). The moisture content of 73.0 to 76.8 percent is higher than other traditional meats. A choice rump roast has a moisture content of 57.0 percent; a pork loin, 57.0 percent; and chicken (fryer, light meat without skin), 60.0 percent.
### TABLE 1. Alligator Yield Data

<table>
<thead>
<tr>
<th>Size</th>
<th>Length (ins)</th>
<th>Live wt. (lbs)</th>
<th>Dressed wt. head off (lbs)</th>
<th>Dressed wt. head off @ 35°F after 12 hrs</th>
<th>Skin wt. (lbs)</th>
<th>Waste wt. head, viscera and feet (lbs)</th>
<th>Tail meat cut (lbs)</th>
<th>Leg meat cut (lbs)</th>
<th>Torso meat cut (lbs)</th>
<th>Jaw meat cut (lbs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>56.5</td>
<td>18.1</td>
<td>11.5</td>
<td>11.0</td>
<td>2.9</td>
<td>4.1</td>
<td>3.8</td>
<td>1.5</td>
<td>4.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Medium</td>
<td>77.5</td>
<td>49.4</td>
<td>30.5</td>
<td>30.4</td>
<td>7.9</td>
<td>11.1</td>
<td>10.2</td>
<td>3.9</td>
<td>13.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Large</td>
<td>88.8</td>
<td>83.6</td>
<td>52.4</td>
<td>50.4</td>
<td>12.2</td>
<td>19.0</td>
<td>17.2</td>
<td>6.2</td>
<td>24.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Extra-Large</td>
<td>110.0</td>
<td>262.0</td>
<td>162.0</td>
<td>159.0</td>
<td>34.8</td>
<td>59.7</td>
<td>48.6</td>
<td>20.0</td>
<td>82.9</td>
<td>8.2</td>
</tr>
</tbody>
</table>

### TABLE 2. Percentage Yield on a Live Weight Basis

<table>
<thead>
<tr>
<th>Size</th>
<th>Dressed wt. head off %</th>
<th>Waste - head, viscera, feet and skin %</th>
<th>Tail meat cut %</th>
<th>Leg meat cut %</th>
<th>Torso meat cut %</th>
<th>Jaw meat cut %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>63.3</td>
<td>38.2</td>
<td>21.1</td>
<td>8.3</td>
<td>27.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Medium</td>
<td>61.7</td>
<td>38.6</td>
<td>20.7</td>
<td>7.8</td>
<td>27.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Large</td>
<td>62.6</td>
<td>37.3</td>
<td>20.6</td>
<td>7.4</td>
<td>29.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Extra-Large</td>
<td>61.8</td>
<td>36.0</td>
<td>18.5</td>
<td>7.6</td>
<td>31.6</td>
<td>3.1</td>
</tr>
</tbody>
</table>

### TABLE 3. Composition of Alligator Meat

<table>
<thead>
<tr>
<th>Cut of Meat</th>
<th>Crude Protein</th>
<th>Crude Fat</th>
<th>Moisture</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail</td>
<td>21.3</td>
<td>1.5</td>
<td>76.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Torso</td>
<td>21.1</td>
<td>1.2</td>
<td>73.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Jaw</td>
<td>22.3</td>
<td>1.2</td>
<td>75.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Leg</td>
<td>21.1</td>
<td>1.0</td>
<td>76.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Analysis performed by LSU Feed and Fertilizer Laboratory.
THE BACTERIOLOGICAL QUALITY AND SAFETY OF PASTEURIZED LANGOSTINO TAILS

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INTRODUCTION

Langostinos (Pleuroncodes planipes and/or monodon), while actually crabs, are harvested and eaten like shrimp. These crustaceans are currently being trawled off the coasts of Chile, Peru and Nicaragua. Huss and Asenjo (1976) reported catches off the Chilean coast at depths of 100–400 m, averaging one ton an hour with yields up to 13%.

MATERIALS AND METHODS

In 1978, pilot plant operations were started in Nicaragua to evaluate the feasibility of establishing a Langostino fisheries. This plant implemented a process that was successfully being used in Chile. The basic process involved the following steps:

1. Raw langostinos delivered whole, on ice.
2. Whole product cooked (blanched) by immersion in hot salt solution (near boiling) for 2 minutes.
3. Hand peeled.
4. Washed in ice water.
5. Packed in 6-oz. packages for pasteurization. Packed in 5-pound blocks for freezing, and/or placed on trays for IQF (individual quick freezing).
6. Pasteurized by immersion of the 6-oz. vacuum packaged product in 190°F water for 2 minutes and subsequent chilling in an ice slush before freezing.

RESULTS AND DISCUSSION

The pilot plant was producing an acceptable product that met the FDA guidelines for frozen-cooked langostinos (Table 1). Due to this successful operation, production was expanded to a larger operation. This expanded operation was accompanied with an increased Staphylococcus contamination in the final product (Table 2, initial process). Assistance from Texas A&M University was requested to alleviate the problem of high staphylococci counts. After on-site inspection, the reasons for the staphylococci counts were thought to be: (1) an inability to adequately control the cooking temperature, (2) a lack of personal hygiene, or (3) a combination of 1 and 2.

To produce a product that would once again be within the FDA guidelines, a modified process was developed. This process is presented in Fig. 1. It differed from the initial process by removing the tails and soaking them in a 5% PO4 solution prior to the blanch and pasteurizing for 3 minutes at
190°F. This time and temperature produced an internal temperature in the langostino tails of 83°C for 1 minute. The newly developed process produced counts as presented in Table 2.

TABLE 1. Food and Drug Administration
Administrative Guideline 7408.10
Limits for Frozen-Cooked Langostinos

<table>
<thead>
<tr>
<th>Limit</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform &lt;20/g (MPN) in 20% of subsamples</td>
<td></td>
</tr>
<tr>
<td>E. coli &lt;3.6/g (MPN) in 20% of subsamples</td>
<td></td>
</tr>
<tr>
<td>Staph &lt;3.6/g (MPN) in 20% of subsamples</td>
<td></td>
</tr>
<tr>
<td>APC (at 35°C) &lt;10⁵/g for all subsamples</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2. Bacteriological Data on Nicaraguan Langostinos
Before and After Pasteurization

<table>
<thead>
<tr>
<th>Initial Process</th>
<th>Developed Process</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unpasteurized</td>
</tr>
<tr>
<td>APC/g</td>
<td>1.3 x 10⁵</td>
</tr>
<tr>
<td>Coliforms MPN/g</td>
<td>9.1</td>
</tr>
<tr>
<td>E. coli MPN/g</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Coagulase-positive &gt;1100</td>
<td>210-290</td>
</tr>
<tr>
<td>Staphylococci MPN/g</td>
<td></td>
</tr>
</tbody>
</table>

In 1979, the State of Florida placed in excess of 280,000 lbs. of langostino meat on a "STOP Sale". The product was subsequently seized by the FDA for adulteration under Administrative Guideline 7408.10. After frozen langostino meat had been inspected by the FDA and Florida, the owners requested a third opinion, and we were asked to sample the lots of langostino. We found (1) not all of the meat was packaged as "frozen-cooked"—some of the lots contained 5-lb. raw boxes; (2) some of the samples packed as cooked reflected high counts, similar to those counts of raw langostino; and (3) some of the samples were acceptable under the FDA Guideline.

As a result of these findings, we decided it was necessary to develop a method of determining to what temperature the product had been cooked. This was done by modifying a method used in the smoked ham industry. The technique measures the turbidity of the thaw drip when heated to different temperatures and relates it directly to the temperature at which the meat was previously cooked. Figure 2 shows typical curves of a raw lot and a sample from that lot after pasteurization to an internal temperature of 83°C for 1 minute. Figure 3 presents curves of six lots, of which only one appears to have reached the desired pasteurization time and temperature. With this method of determining degree of pasteurization, it was discovered
Figure 1. Pasteurized Langostino Processing
FIGURE 2. Denaturation curves for langostino thaw drip (lot 25355 raw) before and after pasteurization (85°C).
FIGURE 3. Denaturation curves from six lots of langostinos labeled pasteurized.
FIGURE 4. Denaturation curves on seven sub-samples of langostinos from lot 23985.
that not all lots were homogeneous. Figure 4 illustrates seven denaturation curves from a typical stratified lot. This stratification within lot appeared to be caused by the lots being transportation lots and not production lots.

CONCLUSION

From the analysis of this data we proposed new bacteriological standards (Table 3). The FDA adopted the raw standards but decided to continue to use the Administrative Guideline 7408.10 (Table 1) for cooked langostinos. It should be noted that the adopted raw standards use the method of the International Commission on Microbiological Specification for Food for establishing bacteriological standards. This is a departure from the earlier standards of "all or none".

TABLE 3. Proposed Bacteriological Standards for Raw and Pasteurized Langostino Meat

<table>
<thead>
<tr>
<th>Product Test</th>
<th>No. Samples (m)</th>
<th>Samples Accepted Between m &amp; M (c)</th>
<th>m</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw (blanched) APC (25°C)</td>
<td>5</td>
<td>3</td>
<td>10^6</td>
<td>10^7</td>
</tr>
<tr>
<td>STAPH</td>
<td>5</td>
<td>3</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Pasteurized (80°C) APC (35°C)</td>
<td>5</td>
<td>1</td>
<td>10^5</td>
<td>10^6</td>
</tr>
<tr>
<td>STAPH</td>
<td>5</td>
<td>1</td>
<td>100</td>
<td>200</td>
</tr>
</tbody>
</table>

The final disposition of the frozen langostino meat has not been decided. The recommendations presented in Table 4 have been presented to the FDA.

TABLE 4. Recommendations for lots of frozen-cooked langostinos

<table>
<thead>
<tr>
<th>Recommendations</th>
<th>No. of lots</th>
<th>Wt. (lb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resample</td>
<td>6</td>
<td>64,686</td>
</tr>
<tr>
<td>Reprocess</td>
<td>1</td>
<td>16,956</td>
</tr>
<tr>
<td>Release</td>
<td>7</td>
<td>99,730</td>
</tr>
<tr>
<td>Not fit for human consumption</td>
<td>14</td>
<td>100,404</td>
</tr>
</tbody>
</table>

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IMPROVEMENT OF SEAFOOD QUALITY

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INTRODUCTION

Very few seafood plants have laboratory capabilities in which to monitor the microbiological quality of their products. When microbiological problems occur, plant managers are often not aware of them until their product is inspected by federal and state regulatory agencies. Moreover, the managers of the plants often lack the training necessary to identify and correct potential microbiological problems. This report is a case study of how serious microbiological problems were identified and corrected in one such plant. While this report concerns only one plant, similar situations are known to exist in other seafood processing plants along the Gulf Coast.

The owners of this particular plant had been processing crabs for a number of years. They started with a small processing plant, but in the late Seventies expanded their operation by building a new, much larger facility. Unfortunately, the volume of crabs processed quickly surpassed the plant's capacity. The owners were conscientious and wanted to produce a good product, but they lacked the knowledge and the time necessary to identify and correct sanitation problems. The management was primarily concerned with sales and much of their time was spent on the telephone and with accounting functions. The day-to-day operation of the plant was left largely to the plant foreman, whose main qualification was that he spoke both English and the language of the workers. The employees were predominately refugees who spoke little English.

The plant operated with two shifts. The day shift packed, weighed and packaged the crabmeat. The second shift was responsible for cooking, debacking and washing the crabs. The owners made an effort to supervise the day shift, but no effort was made to supervise the second shift. The workers on the second shift often took advantage of this lack of supervision to use the plant facilities to clean fish for personal use.

The plant owners were not aware that a sanitation problem existed until the United States Food and Drug Administration conducted an inspection. This inspection revealed that the crabmeat leaving the plant had extremely high aerobic plate counts (APC) and fecal coliform levels. Concern over this inspection prompted owners to contact the Seafood Extension Group at Louisiana State University, who in turn enlisted the help of the Food Science Department.

RESULTS AND DISCUSSION

The basic problem areas identified were the lack of (a) employee
awareness, (b) employee supervision, (c) communication between management and employees, and (d) laboratory capabilities.

To increase employee awareness of sanitation, the Seafood Extension Group prepared a slide/cassette presentation on sanitation in the native language of the workers. The slides carefully documented the correct way of handling crabmeat. It was later recommended that the owners hire a plant manager to oversee the workers and to enforce good sanitary practices. This served to increase employee supervision and communication between the owners and the workers. Also, it was suggested that a microbiology laboratory be set up to monitor the microbiological quality of the crabmeat. A Ph.D. student from the Food Science Department was hired to start this program.

In order to assess the extent of the microbiological problems, representative samples of the crabmeat offered for sale were analyzed in the Food Science Department. Microbiological analyses included testing for aerobic plate counts (APC), total coliforms, fecal coliforms and Staphylococcus aureus. All analyses were done by the methods of USFDA (1) and all media were Difco.

The FDA recommends that crabmeat have an APC of $\leq 100,000/g$ and a fecal coliform count of $\leq 50/100$ g. None of the samples tested were within these recommended limits (Table 1). Line samples and careful observation were used to identify critical control points in the plant (Fig. 1). It might be noted that the crab bodies were grossly contaminated before entering the picking room. The boiled crabs had an APC of 1000/g and were free of fecal coliforms. However, fecal coliforms were added during the debaking and washing steps (Fig. 1). The unsupervised second shift admitted to cleaning fish on the debaking tables and they did not sanitize the tables afterwards. In addition, the workers were using cloth gloves for debaking the crabs. This practice added large numbers of bacteria, including fecal coliforms, to the cooked crabs. After the crabs were debacked they were washed mechanically to remove the fat and intestinal material. Unfortunately, the washers often added to the microbial load of the crabs. This was due to the adherence of crabmeat to the frame and the presence of a protein film on the interior walls.

<table>
<thead>
<tr>
<th></th>
<th>Aerobic plate count/g</th>
<th>Total coliforms/100g</th>
<th>Fecal coliforms/100g</th>
<th>Staphylococcus aureus/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Meat</td>
<td>$3.2 \times 10^6$</td>
<td>2-8000</td>
<td>15000</td>
<td>5</td>
</tr>
<tr>
<td>Claw</td>
<td>$1.0 \times 10^6$</td>
<td>2-2000</td>
<td>7600</td>
<td>125</td>
</tr>
<tr>
<td>Crab Fingers</td>
<td>$5.5 \times 10^6$</td>
<td>4300</td>
<td>4300</td>
<td>50</td>
</tr>
</tbody>
</table>

Log average of 2 samples.

After washing, the crabs were placed into baskets and stacked into the cooler. The baskets were not being sanitized before putting the washed
**FIGURE 1.** Microbiological Analyses of Crab Processing Operation Before Corrective Measures Were Initiated.

A small amount of crabmeat was taken from several pickers and combined to make one sample.
crabs into them and visual inspection revealed pieces of crabmeat and fish scales adhering to the sides of the baskets. The new plant manager quickly cleared up these problems. Plastic gloves were substituted for the cloth debacking gloves and a chlorine dipping solution was provided. The plant owners provided platforms for the baskets and the washer was thoroughly cleaned.

The cooler proved to be the main contributor to the microbiological problem. After being washed in hot water, the baskets of hot moist crabs were stacked in the cooler. The hot moist aerosol from the crabs caused the evaporators to ice up, thus restricting the air flow in the cooler. Often 18-20 h would pass before the defrost cycle cut in to thaw the evaporators. During the interim, it was not unusual to see cooler temperatures above 50°F. The water from the crabs would percolate through the crabs and pick-up water soluble nutrients, thus making an excellent bacteriological medium. When the crab bodies left the cooler the next morning, the APC exceeded 1 x 10⁷/g (Fig. 1). This situation was corrected by adding four defrost cycles and by placing shelves in the cooler. After adding the defrost cycles, the cooler temperature was maintained at approximately 32°F. This reduced the APC of the crab bodies leaving the cooler to 1.2 x 10⁵. The fecal coliform counts were reduced from 4300 to 36/100g. We have further suggested that the crabs be cooled in the same baskets they are boiled in and that the debacking and washing steps be done just before picking.

The workers in the picking room were not observing good sanitation practices. The crabmeat was being picked into unsanitized metal bowls and the the workers were not dipping their hands in the sanitizer provided. The workers would pick up to 8 lb of crabmeat before weighing the bowl; this allowed the picked crabmeat to set at room temperature for several hours. In addition, the workers in the weighing room accepted the crabmeat to the nearest pound and returned the excess to the pickers. Thus, that portion might remain in the bowl all day. Finally, the picked crabmeat was being placed in plastic containers which had not been sanitized. These problems were easily solved by closely supervising the workers and requiring them to abide by good sanitary practices. The bowls are now being sanitized after each weighing and each worker is required to empty the bowl every 60-90 min.

The ice proved to be another source of microorganisms. At first it was believed that the water was contaminated, but we later discovered that the unsupervised second shift workers were storing fish and other foods in the ice.

Another unsanitary aspect of the plant was its circulating waste disposal trough. The water circulating through this trough would become saturated with proteins from the crab waste. The circulating motion would cause the solution to foam which would, at times, actually reach the top of the picking tables and contaminate the crabmeat. We recommended that the plant no longer use this system.

After corrective measures were initiated, the fecal coliform counts were reduced to acceptable levels (Fig. 2). The microbiology laboratory in the plant is operational and the crabmeat is being monitored on a daily basis. This laboratory was relatively inexpensive to set up; it
FIGURE 2. Microbiological Analyses of the Crab Processing Operation After Corrective Measures Were Initiated.
only cost about $2,000 to start. The laboratory facilities give the new plant manager feedback on the effectiveness of his corrective measures. The microbiological quality of the crabmeat has been improving steadily. At the time of this writing, most of the crabmeat samples were free of fecal coliforms and the APC levels have been decreasing (Fig. 2).

REFERENCES