USE OF PHOSPHATES WITH PENAEID SHRIMP

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Water is the largest portion, in both volume and weight, in all edible shrimp products. As the main food component, it has a dominant influence on the sensory attributes, shelf-life and value of the shrimp. Mindful of these concerns, commercial practices have evolved to control, add and retain moisture during shrimp harvest, processing, distribution, storage and preparation. How well these current procedures reflect 'good manufacturing practices,' denoted as federal GMP's (21 CFR Part 110) is in question. Excessive water addition could be deemed adulteration resulting in economic fraud for the buyer, while limited water and water loss can compromise the quality, shelf-life and consumer acceptance of the shrimp products.

COMMERCIAL SITUATION

The current growing concerns for moisture levels in shrimp (Food Chem News, 30 Nov 92) coincides with the most significant period of transition for the nation's penaeid shrimp industry. The domestic fisheries have reached steady-state production in terms of available wild resources and they must compete in a market with an increasing amount of cultured penaeid species from international sources. Since 1980 the total annual U.S. commercial shrimp landings have averaged about 200 million pounds whereas the annual imported poundage has increased from 300 million to over 650 million pounds (Source: Heads-off weight equivalents in USDC/NMFS Fisheries of the U.S., 1991). Domestic processors and food service establishments have grown dependent of foreign aquacultured production. Cultured sources are evolving into foreign processing competition with finished and value-added products. As noted for many domestic industries, competitiveness with an international commodity will require more attention to product quality and good manufacturing practices. Likewise, the new emphasis on seafood safety and nutrition must be incorporated in planning for future shrimp commerce in the U.S. The composition of the shrimp products, particularly moisture content, warrant further consideration relative to GMP's from harvest to consumption.

Use of Phosphates

Use of phosphate agents to influence the moisture content in shrimp was a technology borrowed from the red meat and poultry industries. For penaeid shrimp, the initial intent was to help reduce moisture or drip loss during frozen storage and thawing. This intent, although commonly communicated for well over 25 years, was never formally declared or incorporated by a firm, trade association or regulation. U.S. FDA compliance policy guides (i.e., no. 7303.842), product seizures and letters have referred to sodium tripolyphosphate (STP) to control "drip loss", yet there is no formal regulatory position stated for the for the use of phosphates with penaeid shrimp. From a commercial perspective, controlling drip loss is a function that is considered necessary for raw or cooked, shell-on, peeled or breaded shrimp. In time additional product benefits were noted through
consumer expectations and retention of cook yield. Similarly, Crawford (1980) introduced phosphate applications for cold water shrimp, *Pandalus jordani*. The work focused on phosphates as processing aids to assist peeling and retain protein. His intent reflected distinct and unique processing conditions and product characteristics for a different shrimp genus. Possibly the intended use for phosphate applications on shrimp would require declared functionality and possible distinction by differing processes and various shrimp genera.

Through commercial practice, shrimp firms have learned of the consequences in product quality and yield due to changes and movements in moisture content. This experience is not well documented in peer reviewed literature or regulatory documents. Noting the simplicity of the moisture analysis, it is surprising that there are not previous studies to document the consequences of handling on the water content in penaeid shrimp production, processing and storage. Most previous studies and reviews have focused on biochemical, microbial and sensory consequences (Green, 1949; Campbell and Williams, 1952; Carroll et al, 1968; Pedraja, 1970; Flick and Lovell, 1972; Cobb et al, 1973; Cobb et al, 1977; Bottino et al, 1979; Chung and Lain, 1979; McCoid et al, 1984; Chamberlain and Lawrence, 1983; Chang et al, 1983; Krzynowek, 1988; and Chen et al, 1990). Limited work has noted the loss of water holding capacity and hydration capacity during temporary refrigeration for shrimp (Shelef and Jay, 1971 and Chen et al, 1990). Patents have introduced the use of phosphates to reduce moisture losses during processes, freezing and cooking of shrimp (Stone, 1981 and Shimp et al, 1983). These former reports are limited to the segments of processing studied and are dated by recent changes in processing.

Handling procedures for many domestic products have changed. There is a growing dependence on cultured penaeid species and some initial processing in international settings. For example, much of the breaded products rely on peeling (shell removal) by separate firms, domestic and international, designed to afford this operation. Multi-firm handling requires additional bulk storage and reprocessing. Traditional 'packing houses' for fresh and frozen shrimp and some domestic breading operations using more regional wild harvest expect different consequences in product moisture. Their products are still considered 'penaeid shrimp' in U.S. commerce with all other shrimp sources and forms.

Previous work by Gates et al (1985), Williams et al (1981) and Rao et al, (1975) demonstrated moisture migration in breaded shrimp during frozen retail storage. Inability to retain moisture in the edible muscle during the retail case thaw cycles resulted in breading weight in excess of the allowed 'standards of identity'. Eventual decisions concerning the standards for the U.S. breaded shrimp industry will require additional information on product moisture consequences vs. GMP's. The economic implications of any decisions may represent the most immediate and significant impact on the nation's most valued and established sector of seafood processing.

**Moisture Content**

The reported moisture contents for penaeid shrimp have varied from 71.8% to 87.0% (Table 1). This range results from natural variations by species, season, product size, molting stage and method of production (wild vs. cultured); differences in handling, processing and product forms; and
Table 1. Reported moisture content in raw penaeid shrimp (chronological order).

<table>
<thead>
<tr>
<th>Sample Description and Location</th>
<th>Moisture Content (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>brown shrimp</td>
<td>76.8</td>
<td>Thompson (1964)</td>
</tr>
<tr>
<td>white shrimp</td>
<td>78.2</td>
<td></td>
</tr>
<tr>
<td>1 sample/species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pascagoula, MS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>brown shrimp, Pascagoula, MS</td>
<td>70.6-78.2</td>
<td>Thompson, et al (1971)</td>
</tr>
<tr>
<td>6 bimonthly samples pooled over one year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>brown shrimp</td>
<td>74.46</td>
<td></td>
</tr>
<tr>
<td>white shrimp</td>
<td>71.78</td>
<td>Krishnamoorthy, et al (1979)</td>
</tr>
<tr>
<td>pink shrimp</td>
<td>72.51</td>
<td></td>
</tr>
<tr>
<td>Approx. 11 individual shrimp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>avg./species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocean Springs, MS</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P. aztecus, stylifera, monodon, japonicus, duorarum, indicus, schmitti and setiferus</strong></td>
<td>75.6-81.4</td>
<td>Sidwell (1981)</td>
</tr>
<tr>
<td>depending on species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>white shrimp, two 5 lbs. boxes</td>
<td>82.6</td>
<td>Dudek, et al (1982)</td>
</tr>
<tr>
<td>TX &amp; MS, winter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penaeid and Pandalus</td>
<td>75.9</td>
<td>USDA Handbook No. 8 (1987)</td>
</tr>
<tr>
<td>brown shrimp, 5 lbs. retail</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Louisiana</td>
<td>81.63</td>
<td>Krzynowek and Murphy (1987)</td>
</tr>
<tr>
<td>Texas</td>
<td>84.28</td>
<td></td>
</tr>
<tr>
<td>pink shrimp, 5 lbs. retail</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td>82.97</td>
<td>Krzynowek and Murphy (1987)</td>
</tr>
<tr>
<td>Honduras</td>
<td>80.10</td>
<td></td>
</tr>
<tr>
<td>white shrimp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. Carolina</td>
<td>84.12</td>
<td>Krzynowek and Murphy (1987)</td>
</tr>
<tr>
<td>Georgia</td>
<td>82.03</td>
<td></td>
</tr>
<tr>
<td>Texas</td>
<td>81.90</td>
<td></td>
</tr>
<tr>
<td>P. subtilis, Brazil</td>
<td>80.48</td>
<td>Krzynowek and Murphy (1987)</td>
</tr>
<tr>
<td>P. vannamei, Ecuador</td>
<td>79.64</td>
<td>Krzynowek and Murphy (1987)</td>
</tr>
<tr>
<td>P. setiferous, notalis, vannamei, aztecus, duorarum and subtilis</td>
<td>80.0 - 84.0</td>
<td>Krzynowek and Panunzio (1989)</td>
</tr>
<tr>
<td>depending on species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. aztecus, indicus, occidentalis, notalis, vannamei, schmitti, setiferus, and duorarum</td>
<td>78.2 - 82.3</td>
<td>Garrido, et al (1992)</td>
</tr>
<tr>
<td>These analysis from 10-16 composite samples per species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penaeid sp.</td>
<td>78.87%</td>
<td>Personal Communications (1993)</td>
</tr>
<tr>
<td>unreported results from processing firms trained in the use of rapid moisture analysis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
analytical error due to different procedures, misidentification of samples, poor sampling schemes and assumptions. As the data becomes more current the reported moisture contents tend to increase (Table 1). Each report may have satisfied the question of concern for their respective study, but none of these previous studies compared the consequences of moisture relative to GMP's from production through processing and storage. Most studies simply reflect data per one species, most often misidentified or grouped as "penaeid sp.", sampled at one stage of handling. In most instances the product was frozen and held under variable conditions, temperatures and thawing procedures prior to moisture analysis. Simply stated, they are the best data we have to date for penaeid shrimp. Even the most recent data for eight penaeid shrimp species offers limited interpretation relative to GMP's (Garrido et al, 1992). These samples were all obtained prefrozen with 'faith' in the supplier confirmations that they were not processed with any water addition and they were less than 6 months in storage. Any additional history for product handling is unknown. Similarly, the most recent data being reported by current processing firms reflects a very broad range (78 to 87% moisture) that has not been verified in conjunction with current commercial practice.

Also, freezing and cooking can decrease the moisture content in shrimp so as to adversely affect consumer acceptance (Ahmed et al, 1972 and 1973; Webb et al, 1975 and Applewhite et al 1993). Studies have demonstrated that consumers prefer cooked shrimp with a higher moisture content (Webb et al, 1975 and Applewhite et al, 1993). When challenged with boiled pink shrimp (Penaeus duorarum) pre-treated with phosphates for water additions vs. untreated samples, a group of prescreened consumer panelists (n=125) significantly preferred all treated products over the untreated controls (Applewhite et al, 1992 and 1993). Their response rated a significantly higher perceived 'value' or 'fair market price' for the treated shrimp. This recent study demonstrates the addition of moisture to shrimp is not necessarily adulteration relative to consumer expectations. This study lacks a comparison of moisture content as a result of GMP's per current commercial practices and products. Cooking consequences per GMP's and related consumer expectations warrants further assessments.

REGULATORY SITUATION

Phosphates are multi-purpose GRAS substances ("generally recognized as safe"; 21 CFR 182.1810), when used in accordance with good manufacturing practices (GMP's). The GRAS status is based on previous commercial practice and these ingredients do not require a formal, food additive status. As 'GRAS' substances their use relative to methods for applications and amounts are based on the functional effects. There are no approved limits for the amount of phosphating agents that can be used in shrimp. A previous proposal to establish a "... 0.5% level ... as served ..." limit on sodium tripolyphosphates (STP) "... in [to be frozen] fishery products ..." was never approved (Federal Register 18 Dec 1979: 74845). This proposed regulation stated limits as established for phosphating agents used with poultry and red meats (9 CFR 381.147 and 9 CFR 318.7). These USDA regulations specifically state the purposes for use of phosphates are to "decrease the amount of cooked out juices" and "help protect flavor". These USDA regulations and current commercial practice attempts to comply with a guideline of 0.5% phosphates as added to the products. This guideline assumes that all of the premeasured phosphate treatment as added or pumped into the product is completely incorporated. A similar assumption is not directly applicable to shrimp and other seafood muscle systems.
Despite these distinctions for seafoods and related phosphate applications, international standards have adopted the 0.5% (5g/kg) guideline for phosphate use (Codex Alimentarius Commission 1976 and 1992). Likewise, the recent unified standards for European Union Council on Foods (1994) specify a 5g/kg maximum added phosphate level for frozen seafoods. These international guidelines do not offer methodology for distinguishing added from indigenous phosphate content.

The actual phosphate constituent for which the 0.5% limited refers to has confused interpretation and analysis. Researchers (Tenhet et al, 1981) and the Codex Alimentarius Commission (1976) imply the specified STP limit is for residual phosphates expressed as 'P\(_2\)O\(_5\)'. This constituent is a calculated entity based on the official analytical procedures for phosphorus (AOAC, 1990). A more direct interpretation for the total phosphorus level in untreated or non-phosphated penaeid shrimp (Table 2) nearly equals the proposed 0.5% (500mg/100g) limit when expressed as 'P\(_2\)O\(_5\)'. This statement is based on the fact that the phosphate content expressed as P\(_2\)O\(_5\) ring/100g edible shrimp is equivalent to 2.286 times the corresponding phosphorus content. Realizing this situation the recent Codex Alimentarius Commission's revision of the International Standard for Quick-Frozen Shrimp and Prawns (1992) 'doubled' their previous 1976 recommended allowance for phosphates. Likewise, for the most probable interpretation that the FDA proposed 0.5% level was strictly for the complete STP compound, this structure is rapidly degraded in all treated seafoods and offers no guideline for assessing the amount of phosphates used (Tenhet et al, 1981; Storno 1987; Storno et al 1987; Krynowek and Panunzio, 1993 and Heitkemper et al, 1993).

To further confuse the issue there are no reports published that relate moisture content to the amount or type of phosphates used to treat shrimp. Phosphate suppliers have learned that phosphate blends can be more effective than the traditional use of tripolyphosphates alone. Blend pH (alkaline) is the effective feature in terms of moisture retention. The components in these blends (i.e., hexametaphosphates, pyrophosphate) have not been studied to determine the mode and degree of breakdown in treated products to the pyro-and orthophosphate (phosphorus) forms. This information is necessary to guide commercial and regulatory practice.

The total phosphorus (P) levels in penaeid shrimp offer a simple, direct measure by which to judge previous exposure to a phosphating agent (Table 2). Products with no previous history of phosphate exposure appear to have phosphorus contents less than 250 mg/100g. Higher phosphorus levels suggest previous treatments, but the amount of treatment would still require analysis for moisture content and visual inspections (i.e. glassy appearance, especially when cooked, soapy feel and detectable tastes). Unfortunately, these subjective indicators have not been aligned with phosphorus and moisture contents per phosphate treatments, and they would most likely differ by species.

Confusion for the use of phosphating agents in seafoods has lead to the current commercial and regulatory concerns for added water. This issue was initiated by regulatory positions issued to limit the moisture content of sea scallops (FDA, 1991). Realizing attempts to monitor for phosphate residuals offer only evidence for the use rather than the amount used, FDA opted to focus on the primary adulterant of concern, water. Pressured for an interim policy, the FDA and commercial decisions agreed on moisture guidelines that are proving to be inconsistent with natural moisture levels in various scallops and do not reflect current GMP's. This situation has alienated the respective
Table 2. Reported phosphorus levels (mg/100g) in the edible portion of raw penaeid shrimp

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Phosphorus Content</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Average</td>
</tr>
<tr>
<td>P. brasiliensis</td>
<td>235-291</td>
<td>229</td>
</tr>
<tr>
<td>P. aztecs</td>
<td>210-397</td>
<td>295</td>
</tr>
<tr>
<td>P. indicus</td>
<td>169-170</td>
<td>170</td>
</tr>
<tr>
<td>P. styliferus</td>
<td>235-345</td>
<td>325</td>
</tr>
<tr>
<td>P. monodon</td>
<td>235-345</td>
<td>340</td>
</tr>
<tr>
<td>P. japonicus</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>P. duorarum</td>
<td>278</td>
<td></td>
</tr>
<tr>
<td>P. setiferus</td>
<td>187-332</td>
<td>206</td>
</tr>
<tr>
<td>P. aztecs</td>
<td>207-258</td>
<td>235</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penaeid and Pandalus</td>
<td>179-332</td>
<td>249</td>
</tr>
<tr>
<td></td>
<td></td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>depending on species</td>
<td></td>
</tr>
</tbody>
</table>

1 The phosphate content expressed as P$_2$O$_5$ mg/100g edible shrimp is equivalent to 2.286 times the corresponding phosphorus content. A 218mgP/100g shrimp equals 500mg P$_2$O$_5$ mg/100g shrimp.

2 Details for sampling, prior product handling, analytical procedures and proper species identification are lacking in this reference as well as the cited references.
parties, complicated commerce, devalued some shipments and compromised consumer confidence (TSFT Symposium, 1993). A similar consequence is possible for penaeid shrimp unless a comprehensive study of moisture content is conducted prior to establishing meaningful and useful guidelines. The moisture content of untreated, raw penaeid shrimp has been reported to exceed the 80% moisture level established to distinguish untreated sea scallops, Placopesten magellanicus.

FDA must also consider the nutritional inferiority of phosphate treated products due to possible water 'dilution' of essential nutrients. As a matter of policy, FDA has adopted an 80% rule for purposes of determining nutrition equivalency (21 CFR 101.9, e, 4). This concern is linked to nutrition labeling and possible determination for imitation foods. It is not clear how nutritional equivalence would apply to raw shrimp when they are not fabricated from new technology. Since raw shrimp is not a 'new food' fabricated from new technology (38 Fed. Register 2138 - 1/19/73 and 20702 - 8/2/73) the primary concern would be total percent indigenous protein. This concern is linked to requirements for nutrition labeling. Data reflecting on the protein consequences due to phosphate treatments for penaeid shrimp are lacking, especially for cooked shrimp.

Another questionable regulation used to control the composition of shrimp products is the standard of identity for breaded shrimp (21 CFR 161.175 and 161.176). The first notice for development of this standard was initiated by the National Shrimp Breaders Association with the National Fisheries Institute in 1961 (Fed. Register 3/31/61, page 2723). These dated regulations differ from similar standards of identity for foods in that they do not declare or emphasis things or ingredients that may be used or included in the shrimp. For example, the standard of identity for canned shrimp (21 CFR 161.173) specifies optional ingredients that may be used with this product form. FDA responds to comments on the standard of identity for canned shrimp specifically stated sodium tripolyphosphate was not a suitable ingredient. In contrast, these breaded shrimp standards are concerned with breading, breading ingredients that are suitable, related product nomenclature and analytical methodology to determine percent breading. This FDA opinion and some industry interpretations have assumed these standards exclude the use of phosphating agents. FDA's opinion was specifically stated in their first draft of the Fish and Fishery Products Hazards and Controls Guide (FDA, 1994) which accompanied their proposed HACCP regulations (Fed. Register 59/19:4142, 1/28/94). These assumptions may lie in previous negotiations and understandings, but it is not evident in the language of the standard of identity.

'Shrimp' as defined in the standards of identity for breaded shrimp, 21 CFR 161.175 and 161.176, is the "tail portion of properly prepared shrimp of commercial species". 'Properly prepared' is not defined except for reference (sections c) to the optional product forms [cuts] and the breading and batter. Section 'd' infers the suitable ingredients for the batter and breading by listing non-suitable ingredients (i.e., artificial flavorings, artificial sweeteners, artificial colors and chemical preservatives). In the same section (d) chemical preservatives that are suitable are: 1) ascorbic acid and antioxidant preservatives. Listing ascorbic acid as a suitable ingredient to prevent 'blackspot' (melanosis) on the shrimp is completely inconsistent with commercial practice for penaeid shrimp about the world. Ascorbic acid, either as a breading ingredient or previous shrimp processing aid, does not provide effective prevention of melanosis on penaeid shrimp. As included on the suitable ingredients list in the standard of identity for canned shrimp (21 CFR 161.173), sodium bisulfite and related sulfiting agents are the primary GRAS ingredients used to retard shrimp melanosis. Sulfites are not included in the standards of identity for breaded shrimp despite their prolific use. Although the standards do
not specifically provide for the use of phosphates, they also do not provide for the use of sulfites. The text in section (d) indicates the listed ingredients all relate to the batter and breading. There is no mention of ingredients that may be used or are non-suitable for use with the 'properly processed' shrimp. There is no mention, inference or exclusion of phosphates either as ingredients in batter, breading or properly processed shrimp. According to current GMP's and GRAS status, phosphates have historically been used in processing of shrimp. Likewise, the non-suitable ingredients listed in section d (i.e., artificial flavorings, artificial sweeteners, artificial colors and chemical preservatives) do not refer to phosphates. FDA's proposal rule on the use of phosphates (FR 44/244:74845, 12/8/79) defined phosphates as antioxidants, flavor enhancers, humectants, pH control agents, sequestrants, stabilizers, thickeners and texturizers. Phosphates are not defined or used as preservatives. Current 21 CFR 182, Subpart D, does not include phosphates in the list of chemical preservatives. Phosphates are not used as artificial flavors, sweeteners or colorants. Simply stated, the dated standard of identity for breaded shrimp does not appear to address the use of phosphates and is inconsistent with current domestic and worldwide practice with penaeid and breaded shrimp.

Mindful of potential product abuse or adulteration due to "excessive" moisture addition through the use of phosphates, FDA has recently attempted to discourage use of any substance "added to, or mixed with the product [seafood] to increase its bulk or weight or to reduce its quality, or make it appear of better or greater value than it is (i.e., through adding water to a product by chemical or other means)." This statement is part of FDA's initial seafood HACCP (hazard analysis and critical control point) inspection proposal (Fed. Register 59/19:4142, 1/28/94). Likewise, this concern was reemphasized in FDA's (1994) proposed HACCP regulations and accompanying Fish and Fishery Products Hazards and Controls Guide. Interestingly, this guide, which was not subject to external review with comments, includes the statement (p. 167) that "Sodium tripolyphosphate is not permitted in breaded shrimp or canned shrimp according to FDA's reference to the respective standards of identity (21 CFR 161.175, 161.176 and 161.173)." Although based on confused regulations, current and future regulatory concerns appear to be for the elimination of indiscriminate and possibly any use of phosphate agents with shrimp.

RECOMMENDATIONS

The continued use of phosphates to treat penaeid shrimp remains in question. Documented abuse has rightfully stirred regulatory and commercial attention. Proper use has not been appropriately defined in commercial practice or regulation. The responsibility to resolve this situation in the best interest of commerce and consumers will rely on industry action. Recommendations:

1) Establish baseline data for the composition of raw penaeid shrimp in attempt to better define the moisture and protein content in raw penaeid shrimp.

Published data offers a good start (Table 1), but most of the results can be suspect due to previous handling which may have included exposure to phosphates or other agents. Additional work with 'authentic samples' will be required to account for differences by species, harvest conditions, and post-harvest handling. Mindful of the current trends in shrimp production, authenticity of samples would require international liaison. A specific project to address this issue was rejected by the Saltonstall-Kennedy (NMFS) Grant funds in early 1994.
2) Demonstrate the changes in moisture and protein content in penaeid shrimp as a consequence of frozen storage, processing and cooking.

Published data is limited and often does not account for the influence of phosphating agents. Additional work beginning with authentic, untreated samples must follow through typical processing, storage and cooking regimes. The intent is to demonstrate the need for and influence of water controlling agents in terms of product composition, yield and quality.

3) Declare an intended use and/or function for phosphates in treating penaeid shrimp.

Based on the results from steps 1 and 2, industry must declare functions and related levels or guidelines for use of the phosphating agents. The functions should include concerns for; a) product protection from frozen storage and thaw drip loss, b) moisture, protein and other nutrient retention during cooking, c) reduced cook loss and product dehydration, and d) maintain product quality attributes (i.e. texture and mouthfeel). All of these functions must be demonstrated with actual product through customary processing and handling.

Likewise, this work should include a demonstration of consumer response to actual treated product. Properly designed consumer evaluation studies offer a real measure of quality consequences and value perception. Since FDA's mission is to protect consumer interest, studies are warranted to assess this interest.

4) Declare appropriate guidelines (i.e. moisture and protein levels) to accompany the intended functions and applications.

Industry should attempt to control their own situation through reasonable guidelines recommended for commercial standards and regulation. A raw product weight gain between 5 to 10 percent could be reasonable (Tables 3), but it must be justified by steps 1-3. The corresponding protein changes and influence of cooking must be determined. For example, the ham industry bases phosphate water additions based on a protein fat-free (PFF) measure (Table 4).

Table 4. Product labels for ham as determined by PFF standards.

<table>
<thead>
<tr>
<th>Ham - Cooked and Labeled</th>
<th>Minimum PFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ham</td>
<td>20.5</td>
</tr>
<tr>
<td>Ham, w/Natural Juices</td>
<td>18.5</td>
</tr>
<tr>
<td>Ham, Water Added</td>
<td>17.0</td>
</tr>
<tr>
<td>Ham and Water Product, X% of Weight is</td>
<td>Less than 17.0</td>
</tr>
<tr>
<td>Added Ingredients</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Theoretical changes in the percent composition of raw penaeid shrimp due to weight gained (5 to 20%) through to the addition of water as could be influenced by phosphate treatments. Calculations are provided based on five products with initial moisture contents ranging from 77% to 81%.

<table>
<thead>
<tr>
<th>Percent Weight Gain</th>
<th>Calculated Percent (%) Moisture (M) and Protein (P) Levels.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
</tr>
<tr>
<td>0% Initial</td>
<td>77.0</td>
</tr>
<tr>
<td>5%</td>
<td>78.1</td>
</tr>
<tr>
<td>10%</td>
<td>79.1</td>
</tr>
<tr>
<td>15%</td>
<td>80.0</td>
</tr>
<tr>
<td>20%</td>
<td>80.8</td>
</tr>
</tbody>
</table>

1. Calculations assume an initial 3% ash, fat and carbohydrate content and no loss of protein. All added weight based on added water content.
REFERENCES


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DETERMINATION OF TRIPOLYPHOSPHATE AND RELATED HYDROLYSIS PRODUCTS IN PROCESSED SHRIMP

National Forensic Chemistry Center
U.S. Food and Drug Administration
Cincinnati, Ohio

The Food and Drug Administration is responsible for regulating the use of polyphosphates in seafood. Sodium tripolyphosphate is the most commonly used polyphosphate in the processed shrimp industry; and current regulations limit its use in accordance with Good Manufacturing Practice. The potential exists for economic fraud through mislabeling or excessive use of tripolyphosphate. At this point, the Agency is more concerned with excessive water uptake in treated samples than the amount of phosphate found in the product. However, there is a need for methodology which can 1) provide confirmation of the presence of tripolyphosphate in shrimp and 2) correlate the results obtained for added water with the use and/or abuse of tripolyphosphate.

The determination of total phosphorus by ICP-AES or some other suitable technique will not allow for an accurate and reliable means of regulating polyphosphate use in shrimp because of the widely varying amounts of P found in shrimp naturally [7]. Therefore, it is necessary to analyze for individual polyphosphate species such as tripolyphosphate and pyrophosphate. There are a number of reports dealing with the determination of polyphosphates in detergents by high performance liquid chromatography and ion chromatography [1-4]; however, thin layer paper chromatography often has been used in investigations involving polyphosphates in sea foods [6,8].

The regulation of polyphosphate use is complicated by the fact that polyphosphates are known to undergo hydrolysis. Unfortunately, polyphosphate hydrolysis is catalyzed in shrimp (as well as in other meats) due to the action of tissue phosphatases [5,6,8]. One mole of tripolyphosphate hydrolyzes to one mole of orthophosphate and one mole of pyrophosphate, while one mole of pyrophosphate hydrolyzes to two moles of orthophosphate [5,6,8].

The development of a successful regulatory method for tripolyphosphate in seafood will depend on a number of factors. Of primary concern, is minimizing the amount of hydrolysis which takes place during sample treatment, storage, and analysis. In the case where a significant amount of orthophosphate is formed due to hydrolysis, development of an effective method may be impossible because of high naturally occurring orthophosphate levels. Additionally, it must be shown that higher concentrations of tripolyphosphate and pyrophosphate found in shrimp correlate with greater water retention. Teknet and co-workers investigated the stability of STP in treated shrimp during frozen storage using P32 labeled tripolyphosphate and thin layer chromatography[8]. This
study showed that some breakdown occurs during treatment; however, the tripolyphosphate concentration remained essentially constant over the period studied (2 - 10 weeks frozen storage). Pyrophosphate and orthophosphate concentrations were not found to stay constant during the same period of frozen storage.

In another study, Reddy found that tripolyphosphate and pyrophosphate concentrations were stable over a 10 month period when stored at -25°C [6]. This study also utilized thin layer chromatography to show that the rate of hydrolysis increased with increasing temperature (5, 10, 25, and 35°C). Additionally, the tissue enzymes responsible for catalyzing hydrolysis in uncooked shrimp were characterized.

Progress towards the development of an ion chromatographic method for the determination of tripolyphosphate and pyrophosphate in processed shrimp will be discussed in this communication.

MATERIAL AND METHODS

Apparatus

The ion chromatograph used in this work consisted of a Dionex Automated Sampler Module, Gradient Pump Module, Liquid Chromatography Module-3, Reagent Delivery Module and Variable Wavelength Detector Module-II. Data collection and reduction were accomplished using a Dionex Advanced Computer Interface and 386/25 microcomputer equipped with AI-450 chromatography software. A Dionex IonPac AS7 anion separator column (4 X 250 mm) and IonPac NG1 guard column (4 X 50 mm) were used.

Operating Conditions

The chromatographic mobile phase was 70 mM nitric acid [HNO₃] at a flow rate of 0.5 mL/min. Detection of the phosphate species utilized post-column reaction with 1 g/L ferric nitrate (Fe(NO₃)₃·9 H₂O) in 2 % (V/V) perchloric acid [HClO₄] at a flow rate of 0.5 mL/min. The post-column reagent was mixed with the effluent from the IC column using a mixing tee and a Dionex 500 μL packed reaction coil. Ultraviolet detection of the reaction products was made at 330 nm. The injection volume was 100 μL.

Standards

Food grade sodium tripolyphosphate [Na₅P₃O₁₀] was obtained from Monsanto (St. Louis, MO). Sodium pyrophosphate decahydrate [Na₄P₂O₇·10 H₂O] was obtained from Aldrich (Milwaukee, WI); and sodium phosphate monobasic [NaH₂PO₄·H₂O] was obtained from EM Science (Gibbstown, NJ).

Individual stock standards containing either 1,000 or 10,000 μg/g of analyte were prepared in distilled deionized water (DDW) and stored in HDPE bottles. When stored at room temperature, these stock standards were not found to be significantly affected by hydrolysis for at least 30 days. Working standards were prepared daily in the concentration range 5 - 100 μg/g.
Sample Preparation

Samples used in this work were various commercially treated products from a single shrimp processor. The samples obtained were packaged and ready for sale.

Frozen samples were placed in zip-lock plastic bags and thawed in cool water. Tails and shells were removed (when necessary). A representative sample (generally 15-20 shrimp) was then composited using a Cuisinart Food Processor (Greenwich, CT). Accurately weighed 0.5 g portions of the composite were then placed in 60 mL HDPE bottles and diluted by a factor of 100 (w/w) with DDW. After thoroughly shaking for approximately 30 minutes on a mechanical shaker, the samples were centrifuged to separate the heavier particulate (the centrifugation step was skipped in later analyses). The analytical sample was then prepared by passing a portion of the extract through a 0.2 or 0.45 μm Nylon 66 syringe filter (Alltech, Waukegan, IL) and an activated 300 mg C-18 sample preparation cartridge (Alltech) in series. The first 2 mL's of sample through the filter series was discarded. The C-18 sample preparation cartridges were activated by passing approximately 10 mL of methanol followed by 10 mL of DDW.

RESULTS AND DISCUSSION

Chromatography and Quantitation

The ion chromatographic method used in this work has been described elsewhere for the analysis of sequestering agents in detergents [3]. The method is well suited for the determination of tripolyphosphate and pyrophosphate; however, orthophosphate elutes very near the void. This limits the linear working range of orthophosphate and makes it susceptible to interferences from unretained sample components. Non-linearity is commonly encountered in ion chromatography when a peak is eluted in the water dip.

It is difficult to obtain sodium tripolyphosphate in a relatively pure form. Generally a small amount of pyrophosphate is present. Because of this, it is best to calibrate the system using single component standards. For quantitative work it is necessary to assay the tripolyphosphate standard. This was done by first determining the concentration of pyrophosphate in a ~500 μg/g tripolyphosphate standard. The total amount of phosphorus in the tripolyphosphate standard is then determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Finally, the assay is completed by calculating the amount of P due to tripolyphosphate by subtracting the amount of P coming from pyrophosphate (as determined by IC) from the total amount of P (as determined by ICP-AES). Using this method it was determined that our food grade tripolyphosphate standard contained 92 % (w/w) tripolyphosphate. It should be noted that orthophosphate was not detected above the detection limit in either pyrophosphate or tripolyphosphate 500 μg/g standards.

Peak area responses were used in quantitating the phosphate species. The RSD of peak area response for 12 replicate injections of a mixed standard containing 10 μg/g pyrophosphate and 25 μg/g STP were found to be 0.9 % and 1.2 %, respectively. A linear working range of 0.5 to 50 μg/g
pyrophosphate was established. While for tripolyphosphate, the linear range was 10 to 500 μg/g and for orthophosphate a linear range of 10 to 100 μg/g was established. Table 1 summarizes the figures of merit obtained for this separation.

Results for Cooked Product

Figure 1 shows the chromatogram obtained for a commercial sample of cooked shrimp which was treated with tripolyphosphate by the processor. Ortho-, pyro-, and tripolyphosphate were found in this particular sample. In addition, an unidentified peak at a retention time of 6.1 minutes is present in most shrimp samples analyzed. To date, we have not identified this peak. We have demonstrated that the peak is not sulfate based on a sulfate spike. In addition, preliminary results obtained using an inductively coupled plasma atomic emission spectrometer as a second IC detector (in series with the UV detector), indicate that the unknown peak also does not contain P. No peak was detected at 6.1 minutes when monitoring the 214.9 nm P line.

As a practical note, tripolyphosphate is less strongly retained as the number of shrimp sample injections increases. After the peak moves out of an acceptable window of integration, the column can often be cleaned up by running several column volumes of 200 mM nitric acid. Also, it was found that a 5 mL DDW rinse of the injector after every sample significantly minimized sample carryover problems.

<table>
<thead>
<tr>
<th>Table 1. Figures of Merit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Retention Time (min)</td>
</tr>
<tr>
<td>Linear Range (μg/g)</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
</tr>
<tr>
<td>Log-Log Slope</td>
</tr>
<tr>
<td>Short Term Precision (RSD)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

nd = not determined
Figure 1. Chromatogram obtained for a cooked processed shrimp sample. Peaks: (1) orthophosphate; (2) pyrophosphate; (3) unknown; (4) tripolyphosphate.
The method was evaluated for cooked shrimp initially because of fewer problems with hydrolysis. The action of cooking the product apparently slows enzymatic hydrolysis significantly, as will be discussed later. The reproducibility of peak area response for 10 replicate injections of untreated shrimp spiked to contain 25 µg/g pyrophosphate and 50 µg/g tripolyphosphate was found to be 1.0 % and 1.7 % RSD, respectively. Spike recoveries for untreated cooked shrimp were measured by weighing out a sample of homogenized shrimp and spiking with an aliquot of a 10,000 µg/g stock standard. Recoveries for both pyrophosphate and tripolyphosphate were generally acceptable (Range 88 % - 106 %). The data is summarized in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Spike Recovery Data: Cooked Shrimp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike Level (µg/g), n = 2 unless noted</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td>n = 10, (RSD)</td>
</tr>
<tr>
<td>85</td>
</tr>
</tbody>
</table>

nd = not determined

The results obtained for four cooked processed shrimp samples are shown in Table 3. For each sample, both tripolyphosphate and pyrophosphate were detected. The RSD's for 5 replicate weighings were approximately 6 % for each of the composited samples. In addition, a second sample of medium sized shrimp was prepared and the sample extract was analyzed in triplicate for three consecutive days. The extract was refrigerated between analyses. The results of this study showed little day-to-day variation in concentration of pyrophosphate and tripolyphosphate. Averages of 1195 µg/g pyrophosphate and 3195 µg/g tripolyphosphate were obtained over the three day period with RSD's of 9.1 % and 12.1 %, respectively. The same sample was then stored frozen for two weeks and reanalyzed. Seventy-seven percent of the initial pyrophosphate concentration and 92 % of the tripolyphosphate were recovered.

The samples used in Table 3 were reanalyzed approximately 11 months later. The results of this experiment are shown in Table 4. The last column in Table 4 presents the sum of the concentrations of pyrophosphate and tripolyphosphate expressed in terms of % P₂O₅. For samples A, B, and C the Total % P₂O₅ found after 11 months of frozen storage was 87 %, 89 %, and 103 % of the initial values, respectively. This indicates that very little hydrolysis occurs in frozen storage. Also, it is not known how long these samples were out of frozen storage during the initial analysis.
It is of particular interest that in samples B and C there was very little difference in tripolyphosphate concentration over the 11 month period.

A significant amount of hydrolysis was noted in sample D as indicated by the drop in Total % P$_2$O$_5$ from

Table 3. Determination of Tripolyphosphate and Pyrophosphate in Commercially Treated Cooked Shrimp Samples

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Pyrophosphate Conc. (µg/g)</th>
<th>Tripolyphosphate Conc. (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Small Salad Size</td>
<td>1410 ± 84 (4.8 %)</td>
<td>4101 ± 201 (3.9 %)</td>
</tr>
<tr>
<td>B. Medium 55-65/14 oz.</td>
<td>1340 ± 91 (5.5 %)</td>
<td>3009 ± 179 (4.8 %)</td>
</tr>
<tr>
<td>C. Large 30-40/14 oz.</td>
<td>1306 ± 90 (5.5 %)</td>
<td>2933 ± 194 (5.3 %)</td>
</tr>
<tr>
<td>D. Peel N' Eat 55-65/14 oz.</td>
<td>817 ± 45 (2.3 %)</td>
<td>1375 ± 303 (8.8 %)</td>
</tr>
</tbody>
</table>

Average and 95 % confidence limit reported. Number in parentheses is RSD (n = 5)

Table 4. Comparison of results after 11 months of frozen storage.

<table>
<thead>
<tr>
<th>Sample Type (see Table 3)</th>
<th>Pyrophosphate Conc. (µg/g)</th>
<th>Tripolyphosphate Conc. (µg/g)</th>
<th>Total % P$_2$O$_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 0 mos.</td>
<td>1410</td>
<td>4101</td>
<td>0.46</td>
</tr>
<tr>
<td>A. 11 mos.</td>
<td>2090</td>
<td>2740</td>
<td>0.40</td>
</tr>
<tr>
<td>B. 0 mos.</td>
<td>1340</td>
<td>3009</td>
<td>0.36</td>
</tr>
<tr>
<td>B. 11 mos.</td>
<td>949</td>
<td>2840</td>
<td>0.32</td>
</tr>
<tr>
<td>C. 0 mos.</td>
<td>1306</td>
<td>2933</td>
<td>0.35</td>
</tr>
<tr>
<td>C. 11 mos.</td>
<td>1218</td>
<td>3024</td>
<td>0.36</td>
</tr>
<tr>
<td>D. 0 mos.</td>
<td>817</td>
<td>1375</td>
<td>0.18</td>
</tr>
<tr>
<td>D. 11 mos.</td>
<td>231</td>
<td>690</td>
<td>0.09</td>
</tr>
</tbody>
</table>
0.18 % to 0.09 %. However, for this sample the appearance and odor indicated significant decomposition had taken place. It is not known whether this decomposition occurred during frozen storage or if the sample was not promptly frozen after initial analysis. The presence of pyrophosphate in all of the samples treated with tripolyphosphate indicates the need to include pyrophosphate in any quantitative evaluation of tripolyphosphate use. It is not known if the majority of pyrophosphate encountered in these samples originates from the use of tripolyphosphate which contains large amounts of pyrophosphate or if a significant amount of hydrolysis has taken place during treatment and storage. However, the preliminary results obtained for Total % P$_2$O$_5$ as described above, warrant further study. It is acknowledged here that any hydrolysis which has occurred produces orthophosphate. The inability to distinguish between the additional orthophosphate and naturally occurring orthophosphate induces a certain amount of error in the determination of how much tripolyphosphate was used.

Results for Uncooked Product

Hydrolysis effects are even more of a concern for uncooked product due to enzymatic hydrolysis. This was especially evident when performing spiked recoveries on untreated uncooked shrimp samples. Recoveries of 2700 µg/g pyrophosphate spikes in two different uncooked samples were 112 % and 84 %. However, recoveries of 5000 µg/g tripolyphosphate spikes resulted in recoveries of 84 % and 46 %. In the latter sample a significant amount of pyrophosphate was found. If both pyrophosphate and tripolyphosphate were determined in the latter sample and the sum of the two species is used to calculate recovery (in terms of % P or % P$_2$O$_5$), the calculated recovery increases from 46 % to 96 %.

In a separate experiment the hydrolysis of tripolyphosphate was followed in prepared samples over a period of 3 to 4 days. The same prepared samples were analyzed on successive days and refrigerated between analysis. For two uncooked samples the tripolyphosphate concentration dropped from approximately 2500 µg/g to less than the detection limit in 3 days. While in four cooked samples the tripolyphosphate concentration averaged 94 % of the starting concentration after 4 days.

If it could be shown that tripolyphosphate and pyrophosphate concentrations are stable in frozen storage, it should be possible to evaluate tripolyphosphate use in uncooked product. Obviously the amount of time between sample preparation and analysis would need to be minimized. The major concern then would be how much hydrolysis has taken place during treatment and prior to freezing and how much variation there is in the amount of hydrolysis.

CONCLUSIONS

A method for the determination of pyrophosphate and tripolyphosphate in processed shrimp has been described. The method is generally capable of confirming the use of tripolyphosphate. Recoveries for a 40 µg/g spike of pyrophosphate and tripolyphosphate in cooked shrimp samples were 100 % and 94 %, respectively. An RSD of approximately 6 % was obtained for five replicate weighings of commercially treated cooked shrimp samples.
Quantitative results should preferably be reported in terms of a total concentration of pyrophosphate + tripolyphosphate (expressed as % P₂O₅ or % P). Hydrolysis effects on cooked shrimp during frozen storage were found to be relatively small or non-existent. Whether or not high tripolyphosphate concentrations can be correlated with the amount of added moisture in shrimp needs to be established.

For uncooked processed shrimp the analysis is more difficult because of enzymatic hydrolysis. The same approach as used with cooked shrimp, determining pyrophosphate + tripolyphosphate as % P₂O₅, may prove useful if the amount of hydrolysis occurring during processing and storage is minimal.

REFERENCES


CONSUMER EVALUATIONS OF PHOSPHATED SHRIMP AND SCALLOPS

1LeeAnn Applewhite, 2W. Steve Otwell and 1Laura Garrido
1FL Dept. of Agriculture and Consumer Services,
Bureau of Seafood and Aquaculture
2University of Florida,
Food Science and Human Nutrition Department
Gainesville, Florida

Phosphates are used in the seafood industry primarily to reduce freeze/thaw drip loses that occur during processing and storage. Proper use of phosphates to influence the moisture content in shrimp and scallops is currently in debate relative to regulatory concerns for adulteration vs. commercial concerns for "good manufacturing practices". The objective of the study was to record consumer ratings for various sensory attributes perceived when exposed to phosphated and non-phosphated shrimp and scallops.

Experienced panelist and consumers rated various sensory attributes perceived during encounters with boiled shrimp and broiled scallops previously treated with phosphates. Increasing amounts of water were added to the meats by varying the concentrations of phosphate treatment solutions and exposure times. Initial triangle comparison test for differences in the treatments indicated that over half of the panelist could detect the phosphate treated samples and their ability to distinguish the treated products increased as the moisture content increased. Consumer judgments indicated a preference for the treated product and higher ratings for the phosphated shrimp and scallops were consistent for general appearance, flavor, purchase value and overall quality. The following condensed description of work is based on two papers submitted to Journal of Food Science (Otwell et al. 1993).

METHODS

Pink shrimp (Penaeus duorarum) harvested about Key West, Florida, were headed, peeled and deveined without excessive water contact. Sea scallops (Placopecten magellanicus) were harvested along the New England coast and processed aboard the vessel by researchers from the Virginia Institute of Marine Sciences. Both products were exposed to various phosphate treatments to obtain increasing amounts of water added. The shrimp were boiled using a standard cook procedure the water was brought to a boil, the shrimp added, the water returned to boil and the shrimp boiled 1 minute. The scallops were placed on broiler pans and the temperature monitored until the internal temperatures reached 160°F.

The product evaluations occurred in two stages. The first stage was a discriminative test involving experienced panelists in a more analytical setting (FSHN labs, Univ. Florida). The panelist were food scientists and students familiar with discriminative product and testing and seafood. This test focused on panelists ability to detect differences between treatment variables. Judgements were based on appearance and taste of cooked samples. The procedure was triangle testing where the panelist were asked to distinguish the odd or different sample amongst three cooked samples.
The second stage in organoleptic testing involved 100-125 consumers randomly recruited by phone and prescreened for age, sex, level of income and familiarity with eating boiled shrimp and broiled scallops. The consumers were assembled and briefed before, during and after the product evaluations to assure their understanding of the questions and rating system. Ratings were based on actual product observations and consumption. The consumers were unaware of the test variables and a 1 to 7 point rating scale was used. The ratings were analyzed for mean differences and variance with significance ( =0.05) based on the Walker-Duncan k-ratio test (SAS, 1992).

RESULTS AND DISCUSSION

In general, increasing STP concentration and soak time increased the moisture content in the raw and cooked meats (Tables 1 & 2). In the shrimp, the soaking procedure at 2.0% STP did not impart or retain as much moisture uptake as the tumbling procedures. In tumbling, the higher phosphate treatments increased moisture uptake and retention in the cooked products. In the scallops, the most significant influence was a 6.0 percent change in raw moisture content from the freshwater treated control to the 2.5% STP wash for 24 hours. In both cases, the control samples lost the most water when thawed and cooked while the samples treated with the highest concentrations of phosphates lost the least.

Based on percent correct judgements in the series of triangle comparison tests, the majority of panelist were able to distinguish each phosphated treatment from the controls in both the shrimp and scallops. Product distinction was more obvious for products more heavily phosphated (Tables 3 & 4).

For the consumer evaluations, the participants were asked to rate product appearance and aroma before tasting the product. In all cases, the phosphated product was rated higher than the untreated controls (Tables 5 & 6).

CONCLUSIONS

Consumer evaluations have demonstrated a distinct preference for phosphated shrimp and scallops. The addition of moisture and ability to hold water in cooked product can provide a consumer benefit in terms of flavor and moist mouthfeel. There was no distinct objection to phosphated product appearance, aroma of aftertaste, and additional salt taste was desired. In both cases, the consumers generally felt the phosphated shrimp and scallops meet their expectations and they liked and judged the products to be of high quality and valued more than non-phosphated product.
Table 1. Weigh changes in pink shrimp, *Penaeus duorarum* as a consequence of the phosphate treatments and the standard cooking (boiling) procedures for the discriminative triangle tests.

<table>
<thead>
<tr>
<th>Product Treatments</th>
<th>Avg. Percent (%) weight change</th>
<th>DiscrImEnt</th>
<th>Consumers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw %Uptake</td>
<td>-28.0</td>
<td>-33.9</td>
</tr>
<tr>
<td></td>
<td>%Cook loss (Boiled)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.0</td>
<td>-23.5</td>
<td>-33.9</td>
</tr>
<tr>
<td>Phosphates Treatments</td>
<td>1.5% STP, 15 min.T</td>
<td>10.0</td>
<td>-26.5</td>
</tr>
<tr>
<td></td>
<td>6.0% STP, 30 min.T</td>
<td>17.0</td>
<td>-11.0</td>
</tr>
<tr>
<td></td>
<td>2.0% STP, 30 min.S</td>
<td>5.0</td>
<td>-5.7</td>
</tr>
</tbody>
</table>

1 % uptake and % cook loss are measures in weight change per the original weight of the shrimp before treating with phosphates and before boiling, respectively. The original moisture content in the pink shrimp ranged from 78.5 to 80%. The weight changes primarily reflect changes in the water content.

2 All STP (sodium tripolyphosphate) treatments contained 1.0% sodium chloride. The symbols S=soak and T=tumbles.

Table 2. Resulting moisture contents in sea scallop, *Placopecten magellanicus* as sampled from selected commercial handling and processing procedures, then frozen (-20°), thawed (raw) and broiled (71° internal).

<table>
<thead>
<tr>
<th>Product Treatments After Hand-Shocking</th>
<th>Avg. Percent (%) Moisture Content</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw, Thawed</td>
<td>Broiled</td>
</tr>
<tr>
<td>Control, freshwater wash</td>
<td>79.5a</td>
<td>75.6</td>
</tr>
<tr>
<td>Phosphated Dip, 10% STP, 1 min</td>
<td>81.0 bc</td>
<td>78.0</td>
</tr>
<tr>
<td><strong>Phosphate Washes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4% STP, 20 min.</td>
<td>80.1a c</td>
<td>76.9</td>
</tr>
<tr>
<td>2.5% STP, 5 hr.</td>
<td>80.9 bc</td>
<td>77.7</td>
</tr>
<tr>
<td>2.5% STP, 13 hr.</td>
<td>83.4 d</td>
<td>79.5</td>
</tr>
<tr>
<td>2.5% STP, 24 hr.</td>
<td>84.3 d</td>
<td>80.6</td>
</tr>
</tbody>
</table>

All STP (sodium tripolyphosphate) treatments contained 1.0% sodium chloride.

Average moisture contents (AOAC, 1990) were based on triplicate analyses for subsamples following standard thaw and broil procedures.

Statistical significant differences (p=0.05) are denoted by means labeled with different lower case letters according to Bartlett test for homogeneity and Scheffe test for mean comparisons.
Table 3. Discrimination test results for 24 panelists attempting to identify or judge any noticeable difference between cooked samples of control vs. phosphate treated shrimp.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% Total Correct Judgements¹</th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate Treatments³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0% STP, 30 min S</td>
<td>58</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>1.5% STP, 15 min T</td>
<td>79</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>4.0% STP, 15 min T</td>
<td>63</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>6.0% STP, 30 min T</td>
<td>100</td>
<td>88</td>
<td></td>
</tr>
</tbody>
</table>

¹ A recorded "correct judgement" required proper product identification in both replicates of the control vs. phosphate samples in triangle paired comparisons per each trial.

³ All STP (sodium tripolyphosphate) treatments contained 1.0% sodium chloride. The symbols S=soak and T=tumbles.

Source: Orwell et al. 1993

Table 4. Discrimination test results for 24 panelists attempting to identify or judge any noticeable difference between cooked samples of control vs. phosphate treated scallops.

<table>
<thead>
<tr>
<th>Product Compared to Controls</th>
<th>% Total Correct Judgements¹</th>
<th>% Moisture Content Cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% STP/1 min.</td>
<td>58</td>
<td>52</td>
</tr>
<tr>
<td>4% STP/20 min.</td>
<td>69</td>
<td>66</td>
</tr>
<tr>
<td>2.5% STP/5 hr.</td>
<td>73</td>
<td>71</td>
</tr>
<tr>
<td>2.5% STP/13 hr.</td>
<td>66</td>
<td>83</td>
</tr>
<tr>
<td>2.5% STP/24 hr.</td>
<td>77</td>
<td>85</td>
</tr>
</tbody>
</table>

STP - Sodium tripolyphosphate, plus 1% sodium chloride.

¹ A recorded "correct judgement" required proper product identification in both replicates of the control vs. phosphate samples in triangle paired comparisons per each trial.

Source: Orwell et al. 1993
Table 5. Consumer perception ratings for overall shrimp product 'likableness, quality and value' based on previous evaluations of boiled samples. Value judgements were based on a stated raw product cost of $4.99 per pound.

<table>
<thead>
<tr>
<th>Product Treatments</th>
<th>Likableness Mean</th>
<th>Quality % like</th>
<th>Value Mean</th>
<th>Quality % high qual.</th>
<th>Value Mean</th>
<th>Quality % bargain</th>
<th>Value % bargain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.6a</td>
<td>36</td>
<td>3.3a</td>
<td>2.7a</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate mix¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0% STP, 30 min S</td>
<td>4.7a</td>
<td>62</td>
<td>4.2 b</td>
<td>3.4 b</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5% STP, 15 min T</td>
<td>4.8b</td>
<td>65</td>
<td>4.4 b</td>
<td>3.5 b</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0% STP, 15 min T</td>
<td>5.0b</td>
<td>71</td>
<td>4.6 b</td>
<td>3.8 c</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0% STP, 30 min T</td>
<td>4.7b</td>
<td>59</td>
<td>4.4 b</td>
<td>3.5 b</td>
<td>19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ All STP (sodium tripolyphosphate) treatments contained 1.0% sodium chloride. The symbols S=soak and T=tumbles.

Likableness scale: 1-dislike very much; 4-neither like/dislike; 7-like very much; % like is ratings ≥ 5.

Quality scale: 1-very low quality; 4-neither low/high; 7-very high quality; % high quality is ratings ≥ 5.

Value scale: 1-paid too much; 4-'fair price'; 7-got a bargain; % bargain is ratings ≥ 5.

Statistical significant differences (α = 0.05) are denoted by any two means labeled with different lower case letters.

Source: Otwell et al. 1993

Table 6. Consumer perception ratings for overall scallop product 'likableness, quality and value' based on previous evaluations of broiled samples. Value judgements were based on a provided raw product cost of $6.99 per pound.

<table>
<thead>
<tr>
<th>Product Treatments</th>
<th>Likableness Mean</th>
<th>Quality % like</th>
<th>Value Mean</th>
<th>Quality % high qual.</th>
<th>Value Mean</th>
<th>Quality % bargain</th>
<th>Value % bargain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.9a</td>
<td>64</td>
<td>4.4a</td>
<td>47</td>
<td>3.5a</td>
<td>22</td>
<td>31</td>
</tr>
<tr>
<td>% STP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/1 min.</td>
<td>5.1ab</td>
<td>71</td>
<td>4.7ab</td>
<td>59</td>
<td>3.7ab</td>
<td>20</td>
<td>37</td>
</tr>
<tr>
<td>4/20 min.</td>
<td>5.3ab</td>
<td>74</td>
<td>4.8ab</td>
<td>60</td>
<td>3.9ab</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td>2.5/5 hr.</td>
<td>5.4ab</td>
<td>79</td>
<td>5.0 b</td>
<td>65</td>
<td>3.9ab</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>2.5/13 hr.</td>
<td>5.3 b</td>
<td>75</td>
<td>5.0 b</td>
<td>66</td>
<td>4.0 b</td>
<td>29</td>
<td>37</td>
</tr>
<tr>
<td>2.5/25 hr.</td>
<td>5.5 b</td>
<td>83</td>
<td>2.2 b</td>
<td>66</td>
<td>4.0 b</td>
<td>30</td>
<td>37</td>
</tr>
</tbody>
</table>

STP - sodium tripolyphosphate, plus 1% sodium chloride.

Likableness scale: 1-dislike very much to 7-like very much; % like is ratings ≥ 5.

Quality scale: 1-very low quality to 7-very high quality; % high quality is ratings ≥ 5.

Value scale: 1-paid too much; 4-'fair price' to 7-got a bargain; % bargain is ratings ≥ 5.

Statistical significant differences (α = 0.05) are denoted by any two means labeled with different lower case letters.

Source: Otwell et al. 1993
REFERENCES


COMPUTER-BASED PATHOGEN CONTROL

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The use of computers to monitor and control factors affecting the incidence and proliferation of pathogenic organisms can be an effective tool in safe food production. Pathogen control systems vary from simple hand held instruments that can periodically monitor storage areas, processes, etc. to state-of-the-art computer designs that provide real-time monitoring of the processing and storage of food products. Factors that affect microbiological growth such as thermal application, pH, salinity, moisture, ionic activity, and employee traffic control can be continuously monitored to ensure that food safety standards are satisfied at all times. As a result, these types of control systems provide the processor with an automated and comprehensive approach to minimizing the occurrence of pathogen producing bacteria.

RESULTS AND DISCUSSION

The proper monitoring of temperature during the process and storage of raw and finished product is perhaps the most critical control parameter for pathogenic bacteria. Proper selection and use of temperature sensors are important elements in this control area. The four types of temperature transducers or sensors used in measuring temperature are thermocouples, resistance temperature detectors (RTDs), thermistors, and integrated circuits (ICs).

Thermocouples are the most common thermal measuring devices used because of their relative simplicity and wide support. Two thermocouple wires of different material when soldered together form a thermocouple junction. The measurable potential across the junction corresponds to a temperature. Because of their wide temperature range and available sizes, they are used extensively in many areas for temperature measurement. Thermocouples are point monitoring devices and are not easily affected by conduction or convection temperature sources other than at the junction point. The accuracy of the thermocouple is limited to plus or minus a degree or two. Of concern is the thermocouple relative low voltage which can be affected by electrical noise at different locations. This problem can be eliminated through isolated circuits and shielding. Thermocouples can be mounted in most operations but are usually limited to stationary processes due to the inability of wires to follow the food product through all processes.

RTD's or Resistance Temperature Detectors are more accurate due to a more linear output signal, but more expensive than thermocouples. Since they are more fragile than thermocouples and
require a voltage source for their operation, they are usually mounted in a permanent and protected location. A variety of sizes are available, however because of the larger mass of RTDs self-heating errors may occur.

Thermistors are used where very accurate temperature measurements are required. The thermistor has a fast response time so it can be used to measure very small increments of temperature change over short time intervals. Thermistors need a power source to establish a temperature reading and are limited to certain temperature ranges. Thermistors are also extremely fragile. A good example of thermistor use would be in a laboratory setting for research purposes.

IC's, or integrated circuits are excellent sensors because they are relatively inexpensive and easy to mass produce. The voltage versus temperature algorithm is linear so temperatures can be calculated with accuracy. Some drawbacks of the IC are slowness to measure change in temperature or poor reaction time, constant need for a power source, and limited temperature ranges.

Remote Sensors are self-contained, power supplied micro circuitry, including memory for storing collected data. They contain one or more of the previous mentioned sensors and can be placed in with the product being measured. The sensor can be used to monitor product or process environment temperature as well as temperature abuse during shipping and handling. With remote sensors the complete picture of time versus temperature can be recorded. The stored data can be down-loaded to a personal computer and translated into a usable form with appropriate software.

Infrared imaging is another method of remote temperature measurement. An infrared monitor measures surface temperatures of various products from a distance. The temperature is measured on the emissivity of product surface and therefore is limited to average temperatures as might be found when scanning food production lines. Changes in moisture on the product surface and air volume movement around the product will make minor changes in the temperature readings found in infrared temperature monitoring.

Traditional methods of measuring temperature in microwave ovens are obsolete due to the heating of metal by microwave excitation. Fiber optic measurement allows for the monitoring of temperature when microwaves are used for the cooking process. The fiber optic cable is expensive and is fragile under shock and shear conditions. The datalogger used to interpret temperature data is the heart of the microwave sensing device and that alone is the leading cost factor when comparing other temperature measurement sensors and systems. However this is the only type of system developed for use in microwaves. Due to consumer demand for convenience in cooking, manufacturers are rapidly developing newer measurement devices for microwave cooking.

Presently there are many systems to choose from when designing a complete pathogen control program to monitor temperature, pH, salinity, moisture, and traffic control. They can range from simple hand-held instruments to state-of-the-art computer systems that continuously monitor all phases of manufacturing. These areas would include process control and monitoring, data logging, quality control, and lab automation. Hand-held instruments and dataloggers provide an economical and efficient method when monitoring food processing and storage areas. They can be easily programmed and moved to various
locations to monitor potential problem areas. Due to the popularity and versatility of personal computers, they have evolved into a cost effective and powerful solution for data acquisition and control on the plant floor. In addition, the evolution of control software has made it easy for the average user to integrate measurement and control instrumentation into a system. Under current technology, the user has a choice of four instrument interfacing techniques. They are IEEE 488, also known as the GPIB or HB-1B, data acquisition plug-in boards, RS-232 or serial interface, and the VXI bus. The GPIB bus is available for many sophisticated analytical instruments such as chromatographs and other multiple signal devices. It provides high-speed data transfer (1 Mbytes/sec) and can be used with a variety of computer platforms such as IBM, Apple, and Sun. Plug-in data acquisition boards are rapidly becoming the method of choice due to the low cost and variety of functions. Transducers installed throughout the processing area are connected to the board through signal conditioners. Data collected can be analyzed and displayed as text or graphics, providing the user with a real-time information window of plant floor operations. The RS-232 interface can be found on most simple measurement instruments such as balances and pH meters. It usually is standard on most computer systems and is widely supported by most software programs. VXI was first introduced in 1987 and is regarded as a high performance and rugged interface for industry applications. It combines high speed with a modular design that provides for easy control of advanced measurement systems. All of the above interfaces may be combined on a single system in order to take advantage of instrumentation that will satisfy your cost and performance specifications.

Through the proper selection of sensors and controllers, an automated and comprehensive computer system can be implemented in order to monitor and control those factors that affect the incidence and proliferation of pathogenic organisms in food processing operations.

REFERENCES


RAPID TEST KITS FOR DETECTION OF *LISTERIA MONOCYTOGENES*: A REVIEW

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University of Florida  
Gainesville, FL 32611

*Listeria monocytogenes* is a pathogenic microorganism which can cause serious infections to humans, some of which can be fatal. Estimates suggest that in the United States *L. monocytogenes* is the cause of approximately 1850 cases of serious illness annually, with a fatality rate of 25% (Pinner, et. al., 1992). This pathogen usually affects consumers with a weak immune system, including children, pregnant women, elderly and immunocompromised individuals, causing spontaneous abortion and death (Farber, et. al., 1991). Due to the severity of these cases, U.S. Food and Drug Administration has established a 'zero tolerance' for *L. monocytogenes* in all cooked ready-to-eat food product. Any product found to be contaminated has to be recalled and destroyed. Several million dollars in product loss has resulted due to destruction of contaminated food products.

Ready-to-eat seafoods (i.e., smoked fish, crab meat, cooked shrimp, lobster meat) have shown a significant degree of contamination incidents, according to State and FDA routine plant inspections. Ideally, before these products are released into commerce they should be tested or monitored for *L. monocytogenes* contamination. Currently the official analytical methodology takes 7 to 14 days to confirm the presence or absence of *L. monocytogenes*. This period makes it impossible for routine monitoring when working with highly perishable goods. Seafood product, with a shelf-life of less than 14 days on the average, can not be held awaiting prolonged analytical results. Faster methodologies are needed to better address this concern. Rapid test kits for the detection of Listeria have been developed which offer the industry reliable results in a shorter period of time, 1 to 3 days. Some of these methods do not require extensive training.

There are several test kits commercially available for the detection of *L. monocytogenes* on seafoods. Four of the most popular tests are: VIDAS ImmunoDiagnostic Assay System (bioMerieux Vitex, Inc.), GENE-TRAK Listeria DNA Hybridization Test (GENE-TRAK), LISTERIA-TEK ELISA Test System (ORGANON TEKNIKA) and LISTERTEST Immuno-beads Test (VICAM). A comparison of these methodologies is provided to help direct judgements for use. Subsequent trials will be necessary to verify their use in respective commercial settings.

GENE-TRAK *Listeria monocytogenes* is the only test specific for *L. monocytogenes* and is based on a DNA hybridization test followed by a colorimetric detection. The procedure requires sample enrichment and an overall average testing time of 2 1/2 days. Samples are mixed with modified University of Vermont Medium (mUVM-2) for 24 hrs and then spread onto modified Lithium chloride - ceftazidime agar (mLCA) and incubated for another 24 hrs (Appendix 1A). With an sterile swab, growth from mLCA is suspend in 1 mL of Phosphate Buffered Saline (PBS). The suspension is then heated, lysed and washed. The actual GENE-TRAK assay is preformed on a 5 ml aliquot of
APPENDIX 1: Flow Scheme of the different assays mentioned in the paper.

A) GENE-TRAK

Homogenize 25 g of sample and add 225 mL of mUVM-2 (modified University of Vermont medium)

- Incubate 24 +/- 2 hrs @ 35°C

Transfer to mLCA

- Incubate 24 +/- 2 hrs @ 35°C

Resuspend in 1 mL of Phosphate Buffer Saline (PBS)

- DNAH Assay

- Confirmation of presumptive positive colonies (MOX plates)

B) mini-VIAS

25 g of seafood is homogenized and transferred to 225 mL of Frazer broth base (Difco) and supplement

- Incubate for 22 +/- 2 hrs @ 30°C

0.1 mL of Frazer broth in 10 mL of Frazer broth base

- Incubate for 22 +/- 2 hrs @ 30°C

Heat 15 min @ 100°C

- Transfer 0.5 mL into the VIDAS LIS reagent strip and place it into the automated equipment

Read results
APPENDIX 1 (cont)

C) LISTERTEST

25 g of Sample is mixed with
20 mL of Stomaching Buffer
(massage for 30 sec)

Filter 3 mL of
solution

2 mL
1 mL

Add 100 micro Liters
of immunobeads

Save

2 hrs @ Room Temp.

Place vial on
magnetic rack

10 min

Liquid
Beads

Discard
Wash 2 times
with buffer

Spread beads onto
Lister test media plate

22 hrs @ 37°C

Membrane colony lift

ELIA Assay

Biochemical tests
(Rhamnose fermentation and blood degradation)
on confirmed Listeria

D) Listeria-Tek

Mix 25 g of sample + 225 ml of mFraser broth

24 +/- 2 hrs @ 30°C

0.1 ml into 10 ml of BLEB

24 +/- 2 hrs @ 30°C

1 ml aliquot into a clear glass tube and boil for 20 min

Cool to ambient temperature

Transfer 100 microliters into a 96 well plate
and preform ELISA procedure
the treated sample. If *L. monocytogenes* is present a colored solution will be obtained (GENE-TRAK systems, 1993). This test has the advantage of detecting *L. monocytogenes* directly, thus reducing the analytical time (Appendix 2) (Rodriguez, 1993). It is labor intensive and requires high level of training for the person performing the test. Strict controls for the lysis and heating steps are necessary.

Mini-VIDAS ImmunoDiagnostic Assay System (BioMerieux Vitex) is an automated system of analysis that will detect Listeria spp. from a previously enriched sample. Seafood is enriched for 24 hrs in Frazer broth base plus supplement, then the sample is transferred to frazer broth to be incubated for an additional 24 hrs (Appendix 1B). The enriched sample is heated @ 100 °C for 15 min to lyse the cells, exposing the antigen in the cell for better analytical results and reducing the risk of the technician to infection. An aliquot (0.5 mL) of the enriched sample is placed in a VIDAS LIS reagent strip before positioning in the equipment (Tek Talk, 1993). Results are obtain within an hour. This procedure utilizes Enzyme-Linked Fluorescent Immunoassay (ELFA) as a detection technique and the developed fluorescent product is read by a computer scanner and transformed into positive or negative results (Holloway, J, 1993). This test is not quantitative and takes approximately 2 1/2 days to preform (Appendix 2). With automation one technician could easily preform numerous test simultaneously. The test results are objectives, not depending on worker's interpretation. The only draw-back is that this assay requires high initial investment for necessary equipment and initial reagents.

VICAM ListerTest was developed based on the immunomagnetic capture of viable Listeria spp. cells. Magnetic beads coated with Listeria spp. antibodies are mixed with the sample to be analyze and then separated with the aid of a magnet (Appendix 1C). This mechanical procedure suppresses the need of using an initial selective enrichment. Cells bound to the magnetic beads are washed and plated onto solid selective media for overnight incubation. If growth is present, the colonies are most likely Listeria. Further confirmation is based on Enzyme immunolinked assay (EIA) on a membrane imprint of the plate. Colonies can be picked after been confirmed to be Listeria and determine if any *L. monocytogenes* is present.

The advantage of this test is that confirmed, quantitative result (appendix 2) can be obtained within 24 hrs. This test has been found to be equally sensitive to FDA's official method when analyzing Listeria in shellfish and environmental samples (Jackson, B. J., et.al., 1993). Due to the specificity of the antibody, this testing procedure performs well for detecting Listeria in high bio-burden samples. A disadvantage is labor intensity but this is compensated by the ease of use.

LISTERIA-TEK ELISA Test System (ORGANON TEKNIKA) relays on the specificity of the monoclonal antibodies directed to the Listeria spp. antigen, Enzyme-Linked Immunoassay (Appendix 1D). It is labor intensive (Appendix 2), but several samples can be run at the same time by utilizing the 96 well plate format. This format has the disadvantage of requiring a higher level of training by the operator (Appendix 2). Samples are enriched for 48 hrs, then cells are lysed to expose the targetted antigens. EIA procedure is then preformed to determine presence or absence of Listeria spp. Biochemical tests must be preformed in order to determine the presence of *L. monocytogenes*. 
Appendix 2: Advantages and disadvantages of rapid test kit assays compared

A. - GENE-TRAK

Advantages
- Sensitivity is $1 \times 10^5$ cfu / ml
- Assay will work on high bio-burden
- High specificity
- Results ready in 3 hrs after enrichment
- Low false positive rate

Disadvantages
- non AOAC Approved
- Needs close control on incubation temperatures and procedure
- Positive results will need 48 hrs to be confirmed.

B. - LISTERIA-TEK

Advantages
- USDA Accepted
- Very specific (EIA procedure)
- False positive rate (5.9%)

Disadvantage
- High level of training is required
- Easy cross-contamination during assay
- False negative rate of 8.7%

C. - mini-VIDAS

Advantages
- Specific (EIA procedure)
- USDA accepted
- Low false positive (1.5%)
- Easy to use
- Low level of involvement

Disadvantages
- High initial investment
- Not AOAC approved

D. - LISTERTEST

Advantages
- Specific (EIA procedure)
- Results can be obtained within 24 hrs
- Quantitative analysis
- Low false positive

Disadvantages
- High level of involvement
- Not AOAC approved
- Recovery rate of 30 - 85 % depending on the sample
The Food and Drug Administration official procedure (FDA BAM manual, 1984), as can be seen below, will take 4 days to confirm a negative result and 7 to 10 days for a positive.

Homogenized 25g of final meat sample in 225 mL of Listeria Enrichment Broth (LEB)

(30°C)

24hr

48hr

Streak onto LPM and mMcbride agar

incubate for 24 and 48 hr @ 35°C

Identification of presumptive colonies using biochemical tests (such as blood hemolysis, fermentation of esculin, etc.)

New and better methods are continuously being developed in order to obtain rapid and accurate results which should be comparable to the official FDA testing procedures. Food manufacturers are constantly seeking for faster and more economical ways of ensuring the required level of safety in their product. As shown in this paper, rapid test kits are beneficial to the seafood industry, reducing the analysis time, making possible to ensure good quality and product safety at all times. Since seafood is highly perishable processors can not hold the product for more than 2 - 3 days before distribution.
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