Research Update -

Fish Protein Isolate (FPI)

Jae W. Park
Professor
OSU Seafood Research & Education Center
2001 Marine Drive
Astoria, OR 97103


Herbert O. Hultin
1934 - 2007
RESEARCH GROUPS

University of Massachusetts – Prof. Herb Hultin

MPF Inc – Prof. Herb Hultin

Prof. Lanier

University of Florida – Asst. Prof. Hordur Kristinsson

Oregon State University – Prof. Jae Park

Gyeongsang National University – Prof. Yeung Joon Choi

Suranaree University of Technology – Asso. Prof. Jirawat Yongsawatdigul

West Virginia University – Asst. Prof. Jacek Jaczynski

NC State University – Prof. Tyre Lanier
Early Trials and Recent Improvement

- **Trials in Kodiak and Chile**
  - Acid soluble FPI

- **Iceland**
  - Acid soluble → Alkali soluble

- **MPF Inc**
  - Marinade (FPI Slurry)

- **Proteus**
  - Barrier film for fried items

- **Problems and Control**
  - **Foaming**
    - Probably with acid soluble
    - Not with alkali soluble?
  - **Control process**
    - Bio-reactor (Jaczynski)
  - **Adjustment** – per species, seasonal variation
Introduction

**Surimi (Conventional):** “Refined” and stabilized fish myofibrillar proteins ....

“Refined” means....

**Fish protein isolate (FPI) by pH shift:**

- Solubilize @ high acid/alkaline pH
- Isolate @ the pl
- Adjust to the neutrality
Surimi Process/ Fish Protein Isolate

Conventional method
Mince : cold water = 1: 1 ~ 3
\(\rightarrow\) 2 ~3 Washing cycles
\(\rightarrow\) Dewatering
\(\rightarrow\) Refiner/Screw Press
\(\downarrow\) Lose ~30% (soluble) proteins

Acid- or alkali-aided method
Mince : cold water = 1: 5~9
\(\rightarrow\) pH 2 ~ 3 or 10 ~ 11
\(\rightarrow\) Centrifuge → insoluble parts
\(\rightarrow\) pH 5.5
\(\rightarrow\) Centrifuged - recover precipitates
\(\rightarrow\) Freezing w/ Cryoprotectants
\(\downarrow\) SURIMI / FISH PROTEIN ISOLATE
Surimi Process/ Fish Protein Isolate

Conventional method
Mince : cold water = 1: 1 ~ 3
2 ~ 3 Washing cycles
Refiner/Screw Press
Lose ~30% (soluble) proteins
DENATURATION MUST BE AVOIDED!

Acid- or alkali-aided method
Mince : cold water = 1: 5~9
pH 2 ~ 3 or 10 ~ 11
Centrifuge → insoluble parts
pH 5.5
Centrifuged - recover precipitates
SURIMI / FISH PROTEIN ISOLATE

DENATURATION IS INDUCED!

DENATURATION MUST BE AVOIDED!
Homogenization → Solubilization → Centrifugation

- **Soluble Protein**
- **Neutral Lipids**
- **Sediment**
  - Bone, Membrane lipid, Connective tissue
Homogenization → Solubilization → Centrifugation

- **Neutral Lipids**
- **Soluble Protein**

Membrane lipids removed for **Greater Stability**
Homogenization → Solubilization → Centrifugation → Precipitation → FISH PROTEIN ISOLATE → Freeze with Cryoprotectant → Clean Effluent
### Proteolytic degrade. of homogenate

<table>
<thead>
<tr>
<th>Species</th>
<th>Acid</th>
<th>Alkali</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herring</td>
<td>Yes</td>
<td>Negligible</td>
<td>Undeland et al (2002)</td>
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<tr>
<td>Catfish</td>
<td>No</td>
<td>No</td>
<td>Kristinsson et al (2005)</td>
</tr>
<tr>
<td>Pacific whiting</td>
<td>Yes</td>
<td>No</td>
<td>Choi and Park (2002)</td>
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</table>

### Phospholipids (g/g protein)

<table>
<thead>
<tr>
<th>Species</th>
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<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herring</td>
<td>0.037</td>
<td>0.02</td>
<td>Undeland et al (2002)</td>
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<tr>
<td>Krill</td>
<td>Less lipid removal</td>
<td>More lipid removal</td>
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</tbody>
</table>

### Yield

<table>
<thead>
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<th>Species</th>
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<th>Alkali</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Tilapia</td>
<td>Higher</td>
<td>Higher (≤100 mM NaCl)</td>
<td>Ingadottir (2004)</td>
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<tr>
<td>Atlantic Croaker</td>
<td>Higher</td>
<td>Higher (&gt;100 mM)</td>
<td>Kristinsson and Liang (2006)</td>
</tr>
<tr>
<td>Pacific whiting</td>
<td>Equal</td>
<td>Equal</td>
<td>Thawornchinso. and Park (05)</td>
</tr>
<tr>
<td>Pacific whiting</td>
<td>Equal</td>
<td>Equal</td>
<td>Kim, Choi, and Park (2005)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Equal</td>
<td>Equal</td>
<td>Chen and Jaczynski (2007)</td>
</tr>
</tbody>
</table>

### Gel Color

<table>
<thead>
<tr>
<th>Species</th>
<th>Acid</th>
<th>Alkali</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic Croaker</td>
<td>Higher</td>
<td>Whiter</td>
<td>Kristinsson and Liang (2006)</td>
</tr>
<tr>
<td>Rockfish</td>
<td>Higher</td>
<td>Whiter</td>
<td>Yongsawatdigul and Park (05)</td>
</tr>
<tr>
<td>Pacific whiting</td>
<td>Whiter</td>
<td>Whiter</td>
<td>Choi and Park (2002)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Slightly whiter</td>
<td>Whiter</td>
<td>Chen and Jaczynski (2007a)</td>
</tr>
<tr>
<td>Krill</td>
<td>Significantly whiter</td>
<td>Whiter than conv surimi</td>
<td>Chen and Jaczynski (2007b)</td>
</tr>
<tr>
<td>Menhaden</td>
<td></td>
<td></td>
<td>Perez-Mateos and Lanier (06)</td>
</tr>
<tr>
<td>Sardine/Mackerel</td>
<td></td>
<td></td>
<td>Chaijan et al (2005) - Benjakul</td>
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<tr>
<td>Species</td>
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<td>Alkali</td>
<td>References</td>
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<tr>
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<td>-------------------------------------------</td>
<td>------------------------------------------</td>
<td>------------------------------------------------</td>
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<tr>
<td>Gel Texture</td>
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<td>36 Publications on FPI since 2000</td>
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<tr>
<td>Atlantic Croaker</td>
<td>Similar to conv surimi</td>
<td>Better</td>
<td>Kristinsson and Liang (2006)</td>
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<tr>
<td>Pacific whiting</td>
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<td>Better</td>
<td>Yongsawatdigul and Park (05)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td></td>
<td>Better</td>
<td>Thawornchinso. and Park (07)</td>
</tr>
<tr>
<td>Krill</td>
<td></td>
<td>Better</td>
<td>Chen and Jaczynski (2007a)</td>
</tr>
<tr>
<td>Menhaden</td>
<td></td>
<td>Better</td>
<td>Chen and Jaczynski (2007b)</td>
</tr>
<tr>
<td>Sardine/Mackerel</td>
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<td>Lower than conv. surimi</td>
<td>Perez-Mateos and Lanier (06)</td>
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<td>Oxidation during</td>
<td>Higher</td>
<td>Stable</td>
<td>Kristinsson and Liang (2006)</td>
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<tr>
<td>storage</td>
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<td></td>
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<tr>
<td>Atlantic Croaker</td>
<td></td>
<td></td>
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<tr>
<td>Salt addition on</td>
<td>Lower gel</td>
<td>Lower gel</td>
<td>Choi and Kim (2005)</td>
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<td>gel</td>
<td>Positively/Torsion</td>
<td>Positively/Torsion</td>
<td>Ingadottir (2004)</td>
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<tr>
<td></td>
<td>Negligible</td>
<td>Negligible</td>
<td></td>
</tr>
<tr>
<td>Croaker/J Mackerel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tilapia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlantic Croaker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sardines, Squid</td>
<td></td>
<td></td>
<td>Mexico – Pacheco, Garcia’s group</td>
</tr>
</tbody>
</table>
Protein Recovery (Yield) – Pacific whiting

![Bar chart showing protein recovery at different pH levels](chart.png)

Kim and Park, 2003

Table 1--- **Protein recoveries** and **lipid reductions** using the conventional surimi process, acid-aided process and alkali-aided process to recover proteins from Atlantic croaker

<table>
<thead>
<tr>
<th></th>
<th>Conventional</th>
<th>Acid-aided</th>
<th>Alkali-aided</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein recovery</strong></td>
<td>57.7%a</td>
<td>78.7%c</td>
<td>65.0%b</td>
</tr>
<tr>
<td><strong>Lipid reduction</strong></td>
<td>16.7%a</td>
<td>38.1%b</td>
<td>68.4%c</td>
</tr>
</tbody>
</table>

Means in each row having different superscript letters are significantly different ($P < 0.05$).

Kristinsson and Liang (2006)
Removal of lipid content in FPI

Table 1. Proximate Analysis\textsuperscript{a} of the Recovered Trout Proteins That Were Solubilized at Different pH Values and Precipitated at pH = 5.50\textsuperscript{b}

<table>
<thead>
<tr>
<th>treatment ( (\text{pH value}) )</th>
<th>moisture (%)</th>
<th>lipid (% dry basis)</th>
<th>protein (% dry basis)</th>
<th>ash (% dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>78.07 ± 0.31 b</td>
<td>18.98 ± 1.75 a</td>
<td>36.78 ± 2.52 c</td>
<td>2.14 ± 0.10 a</td>
</tr>
<tr>
<td>3.0</td>
<td>80.50 ± 0.30 a</td>
<td>18.08 ± 1.60 a</td>
<td>53.81 ± 0.51 a</td>
<td>1.60 ± 0.16 b</td>
</tr>
<tr>
<td>12.0</td>
<td>75.49 ± 0.29 c</td>
<td>8.80 ± 0.92 b</td>
<td>45.42 ± 1.81 b</td>
<td>1.61 ± 0.05 b</td>
</tr>
<tr>
<td>12.5</td>
<td>76.94 ± 0.64 b</td>
<td>10.99 ± 0.39 b</td>
<td>44.28 ± 1.65 b</td>
<td>1.37 ± 0.12 b</td>
</tr>
<tr>
<td>13.0</td>
<td>77.62 ± 0.41 b</td>
<td>9.89 ± 0.47 b</td>
<td>49.34 ± 2.11 ab</td>
<td>2.14 ± 0.22 a</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Data are given as mean ± SEM \((n = 3)\). Mean values in a vertical column with different letters were significantly different (least-squares difference test; \( P < 0.05 \)). \textsuperscript{b} The cryoprotectants (4\% sorbitol, 4\% trehalose, 0.3\% phosphate, wt:wt) were added following protein precipitation, and then the proximate analysis was performed. Proximate analysis of trout processing byproducts: 71.3\% moisture, 71.5\% crude protein (dry basis), 15.2\% total lipid (dry basis), and 13.9\% ash (dry basis).

Chen and Jaczynski (2007)

More lipids were removed at alkaline pH!
**Superior Gelling Properties of FPI compared to Conventional Surimi**

Fracture Analysis – Pacific Whiting

![Bar chart showing breaking force (g) and deformation (mm) for different pH levels. The chart compares FPI and conventional surimi.](Dr. Park’s Lab, 2003)
Dynamic Gel Property – Rock Fish

(Yongsawatdigul and Park, 2004)
Texture of menhaden surimi and FPI

Fig. 1. Comparison of puncture test values for surimi gels made with three surimi types: conventionally washed (CW), acid-solubilized (AC) and alkaline-solubilized (AL). Gels were made with or without 2% NaCl, and with or without 0.2% added microbial transglutaminase (TGase). 90 indicates that gels were cooked at 90 °C for 20 min, 30/90 and 40/90 indicate gels incubated at 30 and 40 °C for 30 min, respectively, followed by cooking at 90 °C for 20 min. Error bars represent the standard deviation of 6-10 samples.

Perez-Mateos and Lanier (2006)
**Superior Gelling Properties of FPI:**

**WHY?**

- **Chemically**
  - Conformational changes in HMM → Better Charge Distribution
  - Partial refolding at neutral pH. The pH shift
    - Increased reactive Thiol group (R-SH)
    - Increased exposure of more Hydrophobic Groups

Kristinsson and Hultin, 2003
Thawornchinsomsobut and Park, 2007
Superior Gelling Properties of FPI: WHY?

Physically

- Enhanced Dispersion of Myofibrillar Proteins
  - **Stronger, more deformable gels** are related to more homogeneous dispersion of myofibrillar proteins
  - Supported by TEM

Sato and Tsuchiya (1992)
Wright and Lanier (2008)
Superior Gelling Properties of FPI: WHY?

Conventional Surimi:

Higher *myofibrillar* protein content,
Lower *sarcoplasmic* protein content

Means

Superior Gelling Properties ?
Superior Gelling Properties of FPI: WHY?

Higher myofibrillar protein content,
Lower sarcoplasmic protein content

Several reports showing Sarcoplasmic proteins contribute positively to gel formation of myofibrillar proteins:

Morioka and Shimizu, 1990
  Morioka et al., 1992
Morioka and Shimizu, 1993
  Nomura et al., 1995
Ko and Hwang, 1995
Kim and Park, 2003
Park and Park, 2007
Effects of Sarcoplasmic Protein (SP) addition to Pollock surim

![Breaking force (g) Deformation (mm)]

Kim and Park (2005)
Figure 3—Cathepsin L activities of samples at various treatments: A: Water-soluble protein after 1-washing cycle; B: Water-soluble protein after 3-washing cycle; C: Supernatants after pH 2.5 treatment; D: Supernatants after pH 5.5 treatment; E: Surimi after 3-washing cycle; F: Surimi from acid-aided processing.

- A: Water-soluble fraction after 1 washing;
- B: Water-soluble fraction after 3 washing;
- C: Supernatant after pH 2.5 solubilization;
- D: Supernatant after pH 5.5 precipitation;
- E: Surimi after 3 washing;
- F: FPI after acid solubilization
### Table 2 – Color values of ground raw material, surimi, and protein isolates (PIs)

<table>
<thead>
<tr>
<th>Sample</th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
<th>Whiteness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surimi</td>
<td>70.4 ± 1.1a</td>
<td>-0.9 ± 0.2a</td>
<td>0.7 ± 0.4a</td>
<td>70.4a</td>
</tr>
<tr>
<td>Acid PI</td>
<td>73.8 ± 0.4b</td>
<td>-3.6 ± 0.2c</td>
<td>5.7 ± 0.3c</td>
<td>72.9b</td>
</tr>
<tr>
<td>Acid PI (skip 1st centrifugation)</td>
<td>75.1 ± 0.3c</td>
<td>-2.3 ± 0.2b</td>
<td>7.9 ± 0.5d</td>
<td>73.8c</td>
</tr>
<tr>
<td>Alkali PI</td>
<td>75.0 ± 0.7c</td>
<td>-3.0 ± 0.2c</td>
<td>0.2 ± 0.4a</td>
<td>74.8d</td>
</tr>
<tr>
<td>Alkali PI (skip 1st centrifugation)</td>
<td>78.4 ± 0.3d</td>
<td>-2.2 ± 0.1b</td>
<td>3.2 ± 0.0b</td>
<td>78.1e</td>
</tr>
</tbody>
</table>

*Means within 1 species having different letters are significantly different ($P < 0.05$).

Yongsawatdigul and Park (2005)

### Table 1 – Color values of samples prepared by various treatments

<table>
<thead>
<tr>
<th>Sample</th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
<th>Whiteness</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>77.63$^c$</td>
<td>2.60$^a$</td>
<td>7.19$^a$</td>
<td>56.07$^d$</td>
</tr>
<tr>
<td>WM</td>
<td>81.22$^a$</td>
<td>1.69$^b$</td>
<td>0.83$^d$</td>
<td>78.74$^a$</td>
</tr>
<tr>
<td>AC</td>
<td>79.06$^b$</td>
<td>1.17$^c$</td>
<td>4.74$^c$</td>
<td>64.84$^b$</td>
</tr>
<tr>
<td>AK</td>
<td>76.20$^d$</td>
<td>-0.04$^d$</td>
<td>5.67$^b$</td>
<td>59.18$^c$</td>
</tr>
</tbody>
</table>

*Means with different letters in the same columns are significantly different ($P < 0.05$).

Kristinsson et al (2005)
Effects of pH on the appearance of Sarcoplasmic Proteins (SP) from Pacific whiting.

**SP (pH 7)**
(No NaCl)

- pH 2
- pH 10
- pH 12

- pH 5.5 (No NaCl)
Effect of salt addition on gelation of FPI

**Figure 7-1.** Shows shear stress values (kPa) of gels produced from white muscle proteins of tilapia. The use of low and high solubilization pH treatment was compared to a three cycle washing treatment (control). Gels were cooked in steel tubes at 80°C for 30 min. The gels were stored in a cold room at 4°C for 48 hours prior to testing with a Torsion Gelometer. Results are mean ± SD. Different capital letters indicate a significant difference (p < 0.01) for treatments without 2% NaCl. Different small letters indicate a significant difference for treatments with 2% NaCl. For each treatment gels with 2% NaCl (w/w) had a significantly higher stress value, except for washed muscle.
Effect of salt addition on gelation of FPI

Ingadottir (2004)

Figure 7-3. Storage modulus (G’) of protein pastes at 5°C before gelation. Results are mean ± SD.
Effect of Salt Addition on Texture of Menhaden Surimi and FPI

Salt addition on FPI:
- Negative on strength

Salt addition:
- Negative or Positive on deformation

Fig. 1. Comparison of puncture test values for surimi gels made with three surimi types: conventionally washed (CW), acid-solubilized (AC) and alkaline-solubilized (AL). Gels were made with or without 2% NaCl, and with or without 0.2% added microbial transglutaminase (TGase). 90 indicates that gels were cooked at 90 °C for 20 min, 30/90 and 40/90 indicate gels incubated at 30 and 40 °C for 30 min, respectively, followed by cooking at 90 °C for 20 min. Error bars represent the standard deviation of 6-10 samples.

Perez-Mateos and Lanier (2006)
Effect of salt addition on gelation of FPI

Conventional Surimi

Acid FPI

Alkali FPI

Kim and Park, 2008
Should FPI be stored frozen with cryoprotectants? At what pH?

(Thawornchinsombut and Park 2004)
Current Commercial Players

**MPF Inc**
- Alkaline and Acid solubilization
- Product development with private industries
- Promote FPI slurry (marinade) injection

**Proteus Industries Inc**
- Acid solubilization only
- Product development with private industries
- Promote FPI to form a barrier film for fried foods → Reduced fat pickup by 25-75%
Current Commercial Players

- **MPF Inc**
  - Alkaline and Acid solubilization
  - Product development with a private industry
  - Promote FPI slurry (marinade) injection

- **Proteus Industries Inc**
  - Acid solubilization only
  - Product development with a private industry
  - Promote FPI to form a barrier film for fried foods
    - Reduced fat pickup by 25-75%

The Future of FPI Technology has to be directed to:

*Fish Protein Isolate to replace Conventional Surimi!*
Are chemicals (NaOH and HCl) used simply as a processing aid?

- pH 5.5: 2 N NaOH
- pH 6.0 ~ 6.5: 2 N NaOH
- pH 7.0: 2 N NaOH
- pH 11: 2 N HCl

FPI (Fish Protein Isolate)
NaOH + HCl = NaCl + H₂O

pH 6.0~6.5 → pH 11.0 → pH 5.5 → pH 7.0

Questions need to be answered:

1. How much NaOH was added?
2. How much HCl was added?
3. Were all chemicals canceled out as salt and water?
4. Was there any residual NaOH unused after neutralizing HCl?
NaOH + HCl = NaCl + H₂O

pH 6.0~6.5 → pH 11.0 → pH 5.5 → pH 7.0

21 CFR 101.100(a)(3)(ii)(b) -

Substances that are added to a food during processing, are converted into constituents normally present in the food, and do not significantly increase the amount of the constituents naturally found in the food.

Therefore, NaOH and HCl are used as a processing aid and no labeling is required.
Summary

1. Proteins were chemically unfolded (denatured?) when solubilized in acid or alkali.
2. Upon the pH adjustment to 7, myosin was partially refolded back.
3. Highly homogenous dispersion improved gelation.
4. Alkaline solubilization gave better texture and reduced lipid content.
5. Acid solubilization gave slightly better color.
6. Higher yield was obtained by both acid and alkali solubilization.
7. No significant effect of frozen storage pH was reported, but cryoprotectants must be added.
Thank You.....