Research Update -

Fish Protein Isolate (FPI)

Jae W. Park

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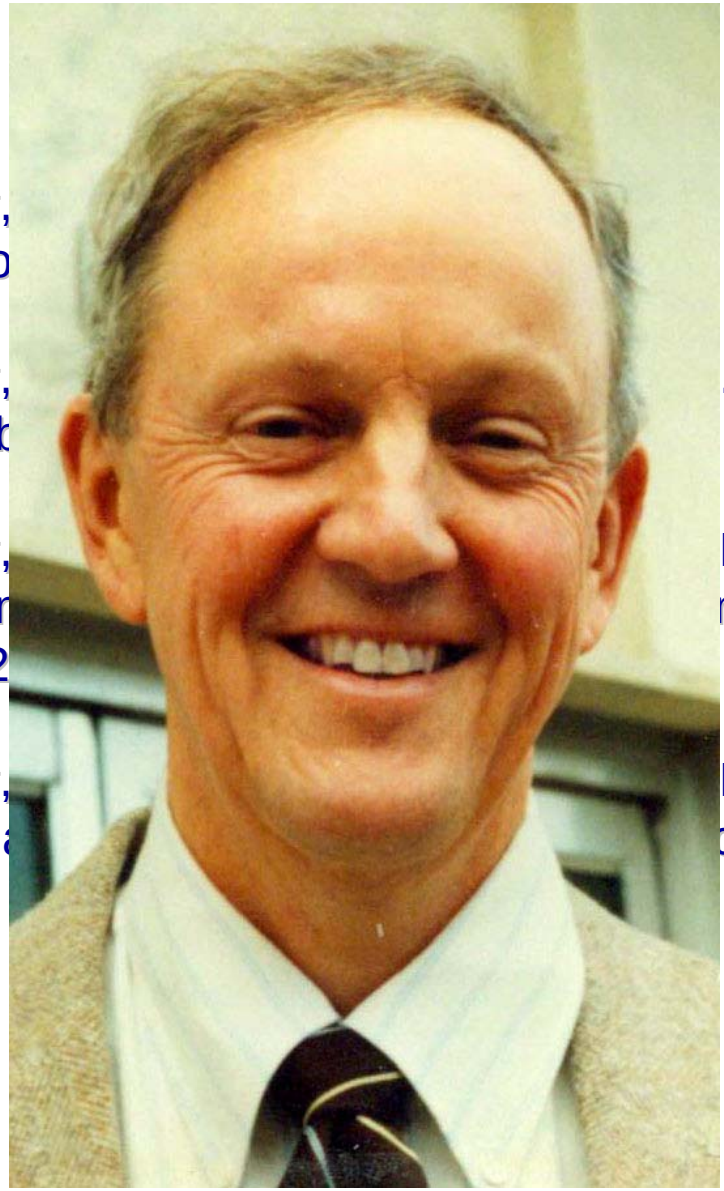
Hultin, H.O. and Kelleher, “Protein composition isolated from a muscle so

Hultin, H.O. and Kelleher, . “High efficiency alkaline protein extraction”. Octob

Hultin, H.O. and Kelleher, B1. “Process for isolating a protein composition fror mposition”. September 11, 2

Hultin, H.O. and Kelleher, B1. “Protein composition and process for isolating a cle source. September 17, 2002.”



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

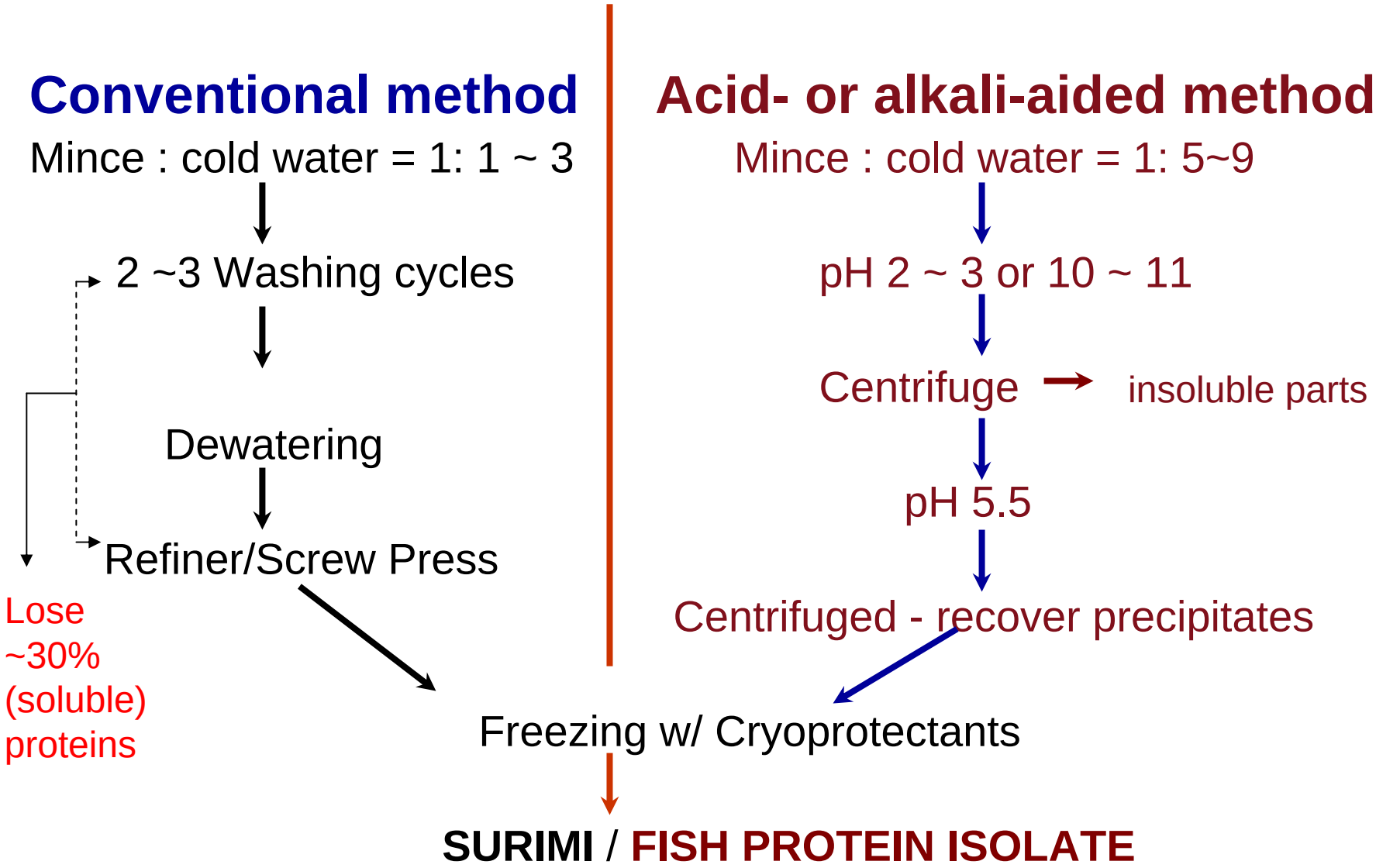
Introduction

- **Surimi (Conventional): "Refined" and stabilized fish myofibrillar proteins ….**

  "Refined" means….

- **Fish protein isolate (FPI) by pH shift:**
  - **Solubilize @ high acid/alkaline pH**
  - **Isolate @ the pI**
  - **Adjust to the neutrality**

Surimi Process/ Fish Protein Isolate



Surimi Process/ Fish Protein Isolate

Conventional method

Mince : cold water = 1: 1 ~ 3

2 ~ 3 Washing cycles

DENATURATION MUST BE AVOIDED!

Dewatering

Refiner/Screw Press

Lose ~30% (soluble) proteins

Acid- or alkali-aided method

Mince : cold water = 1: 5~9

pH 2 ~ 3 or 10 ~ 11

Centrifuge → insoluble parts

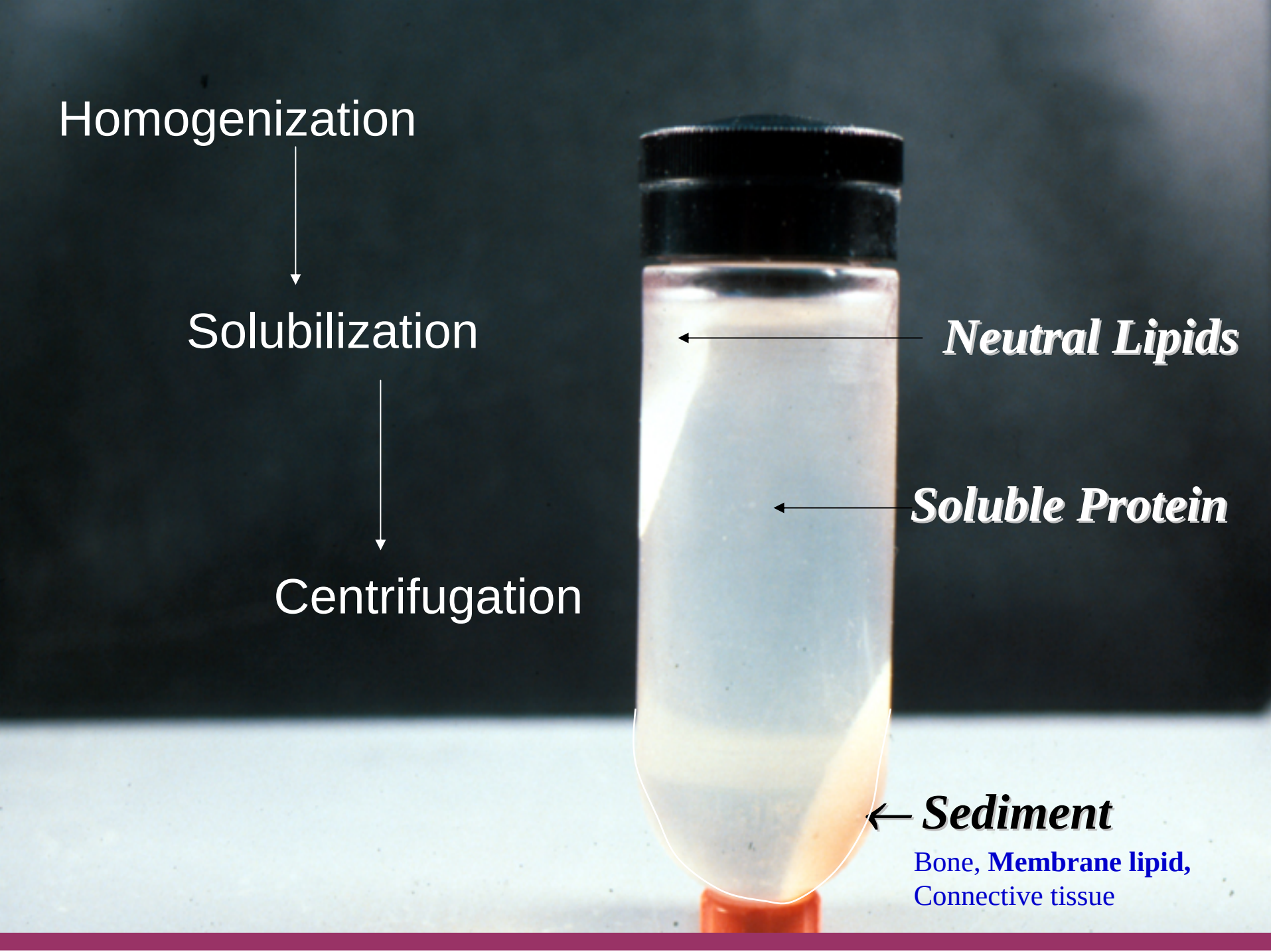
pH 5.5

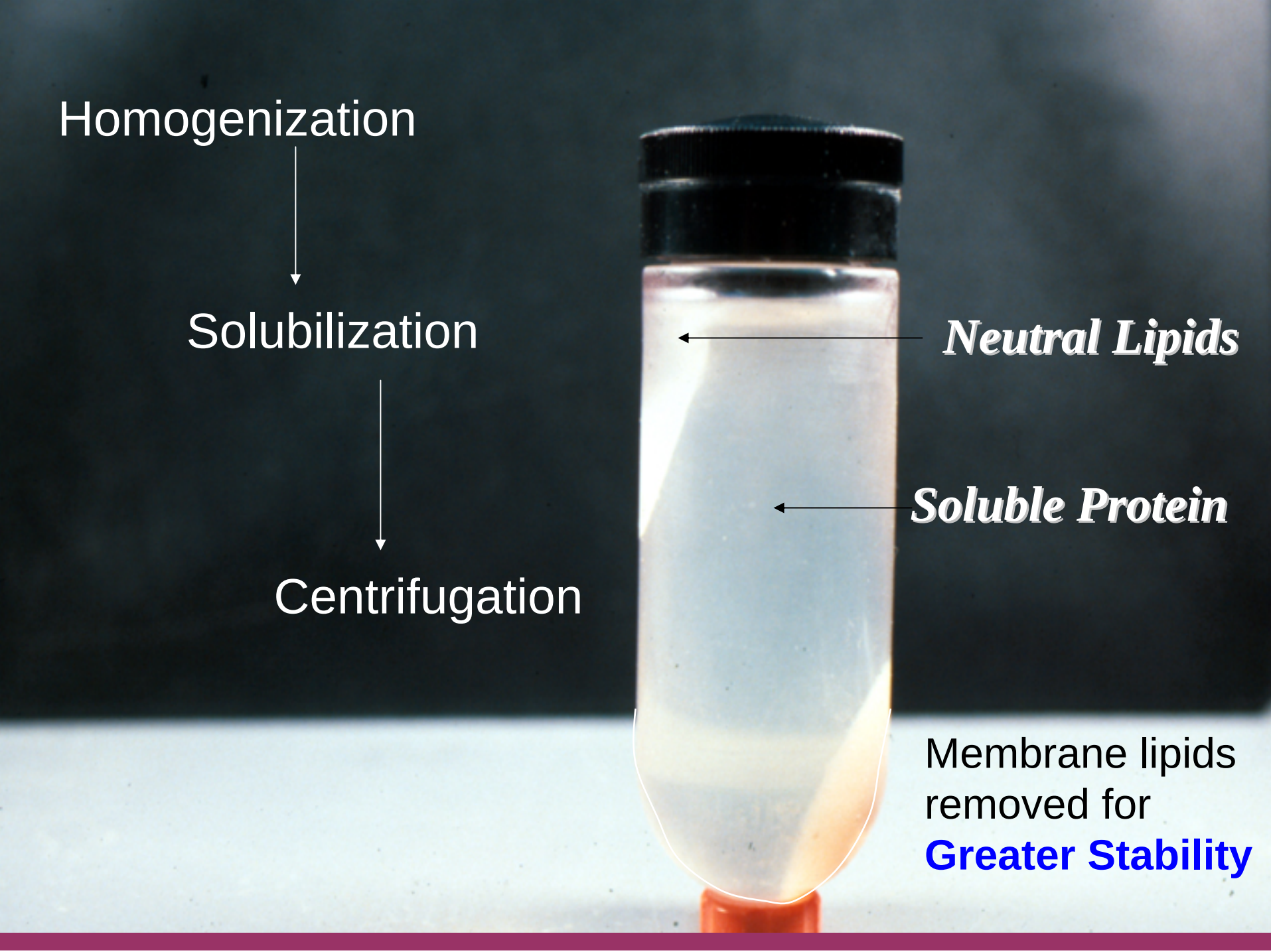
Centrifuged - recover precipitates

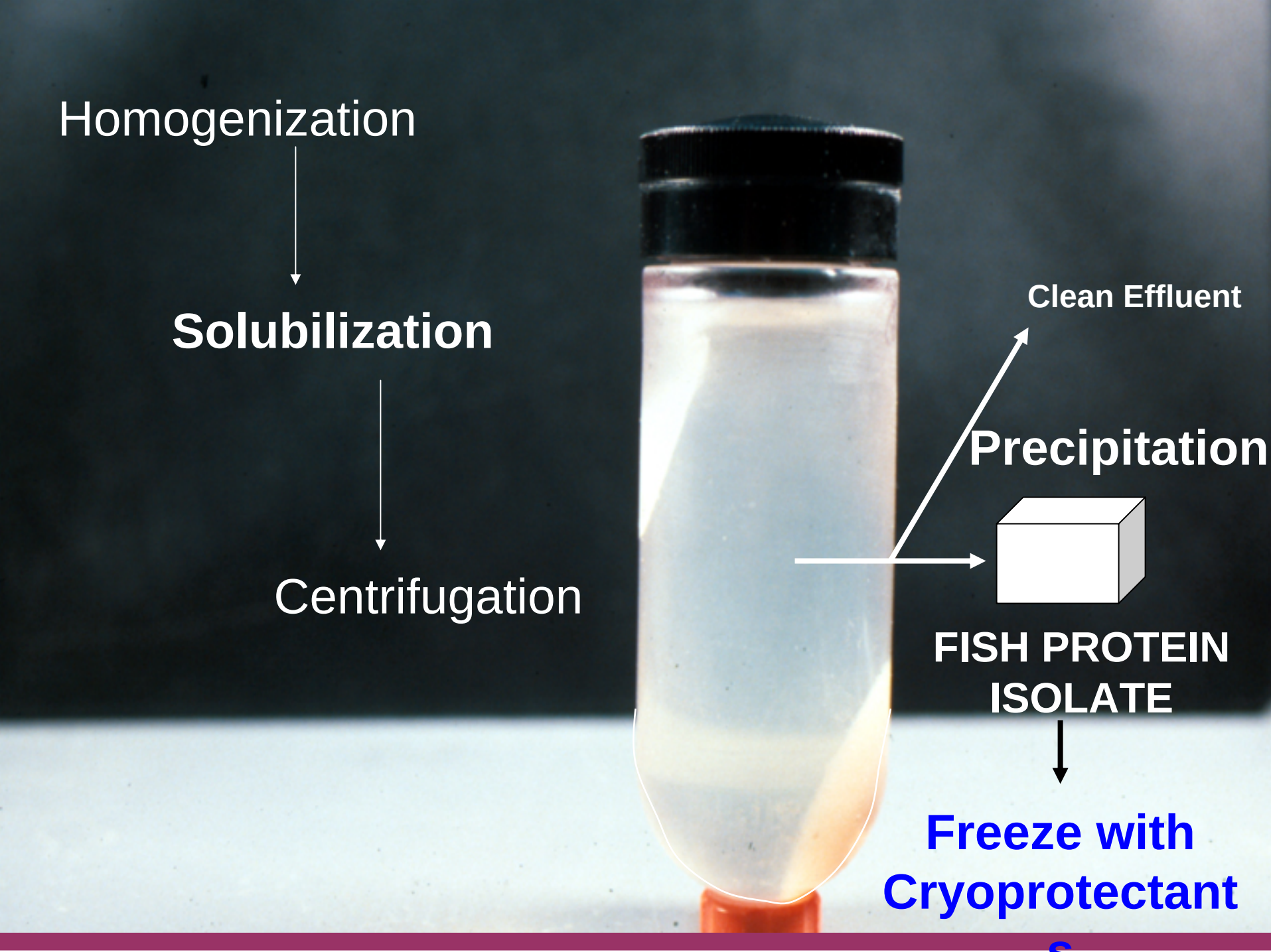
DENATURATION IS INDUCED!

Freezing w/ Cryoprotectants

SURIMI / FISH PROTEIN ISOLATE


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


Homogenization
Solubilization
Centrifugation
Neutral Lipids
Soluble Protein
Membrane lipids removed for
Greater Stability


Homogenization
Solubilization
Centrifugation
Clean Effluent
Precipitation
FISH PROTEIN ISOLATE
Freeze with Cryoprotectant

36 Publications on FPI since 2000

	Species	Acid	Alkali	Reference
Proteolytic degrade. of homogenate	Herring Catfish Pacific whiting	Yes No Yes	Negligible No	Undeland et al (2002) Kristinsson et al (2005) Choi and Park (2002)
Phospholipids (g/g protein) Removal of lipid	Herring Rainbow trout Krill	0.037 Less lipid removal Less lipid removal	0.02 More lipid removal More lipid removal	Undeland et al (2002) Chen and Jaczynski (2007a) Chen and Jaczynski (2007b)
Yield	Croaker/J. Mackerel Tilapia Catfish Atlantic Croaker Pacific whiting Pacific whiting Rainbow trout	 Equal Higher Higher (<100 mM NaCl) Equal Equal	Higher Higher Equal Higher (>100 mM) Equal Equal	Choi and Kim (2005) Ingadottir (2004) Kristinsson et al (2005) Kristinsson and Liang (2006) Thawornchinso. and Park (05) Kim, Choi, and Park (2005) Chen and Jaczynski (2007)
Gel Color	Herring Catfish Atlantic Croaker Rockfish Pacific whiting Pacific whiting Rainbow trout Krill Menhaden Sardine/Mackerel	Equal Higher b* Higher b* Whiter (Lower b*) Darker than conv surimi Slightly whiter Significantly whiter	 Whiter Whiter Whiter Whiter than conv surimi	Undeland et al (2002) Kristinsson et al (2005) Kristinsson and Liang (2006) Yongsawatdigul and Park (05) Choi and Park (2002) Kim, Choi, and Park (2003) Chen and Jaczynski (2007a) Chen and Jaczynski (2007b) Perez-Mateos and Lanier (06) Chaijan et al (2005) - Benjakul

36 Publications on FPI since 2000

	Species	Acid	Alkali	References
Gel Texture	Herring	Equal	Equal	Undeland et al (2002)
	Tilapia		Better	Ingadottir (2004)
	Atlantic Croaker		Better	Kristinsson and Liang (2006)
			Better	Perez-Mateos et al (2004)
	Rockfish		Better	Yongsawatdigul and Park (05)
	Pacific whiting	Similar to conv surimi		Choi and Park (2002)
	Pacific whiting	Equal	Equal	Kim, Choi and Park (2003)
	Pacific whiting		Better	Thawornchinso. and Park (07)
	Rainbow trout		Better	Chen and Jaczynski (2007a)
	Krill		Better	Chen and Jaczynski (2007b)
	Menhaden		Better	Perez-Mateos and Lanier (06)
	Sardine/Mackerel		Lower than conv. surimi	Chaijan et al. (2005) - Benjakul
Oxidation during storage	Atlantic Croaker	Higher	Stable	Kristinsson and Liang (2006)
Salt addition on gel	Croaker/J Mackerel	Lower gel	Lower gel	Choi and Kim (2005
	Tilapia	Positively/Torsion	Positively/Torsion	Ingadottir (2004)
		Negatively/G'	Negatively/G'	
	Atlantic Croaker	Negligible	Negligible	Perez-Matero et al (2004)
	Sardines, Squid			Mexico – Pacheco, Garcia's group

Protein Recovery (**Yield**) – Pacific whiting

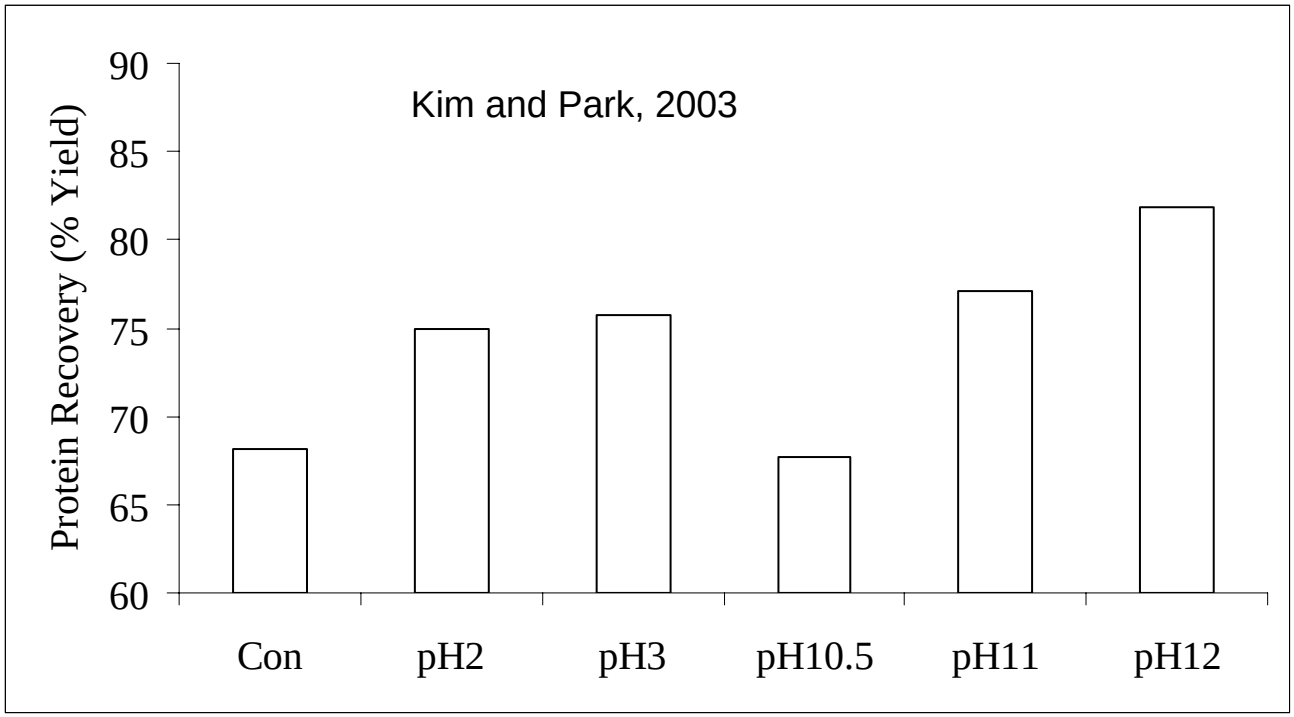


Table 1--- Protein recoveries and **lipid reductions** using the conventional surimi process, acid-aided process and alkali-aided process to recover proteins from **Atlantic croaker**

	Conventional	**Acid-aided**	**Alkali-aided**
Protein recovery	57.7%a	**78.7%c**	65.0%b
Lipid reduction	16.7%a	38.1%b	68.4%c

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[a] of the Recovered Trout Proteins That Were Solubilized at Different pH Values and Precipitated at pH = 5.50[b]

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

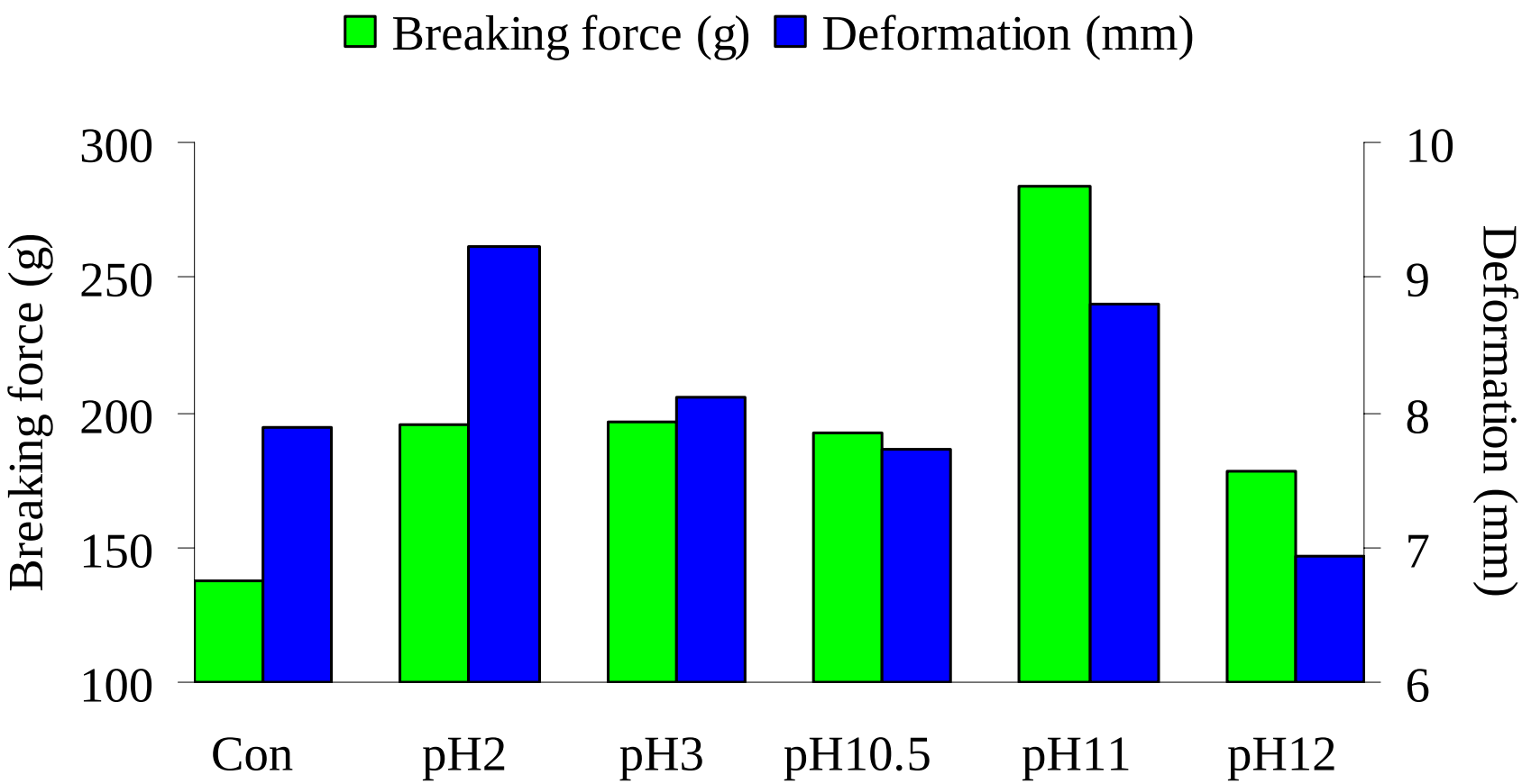
More lipids were removed at alkaline pH!

[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; $P < 0.05$). [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)

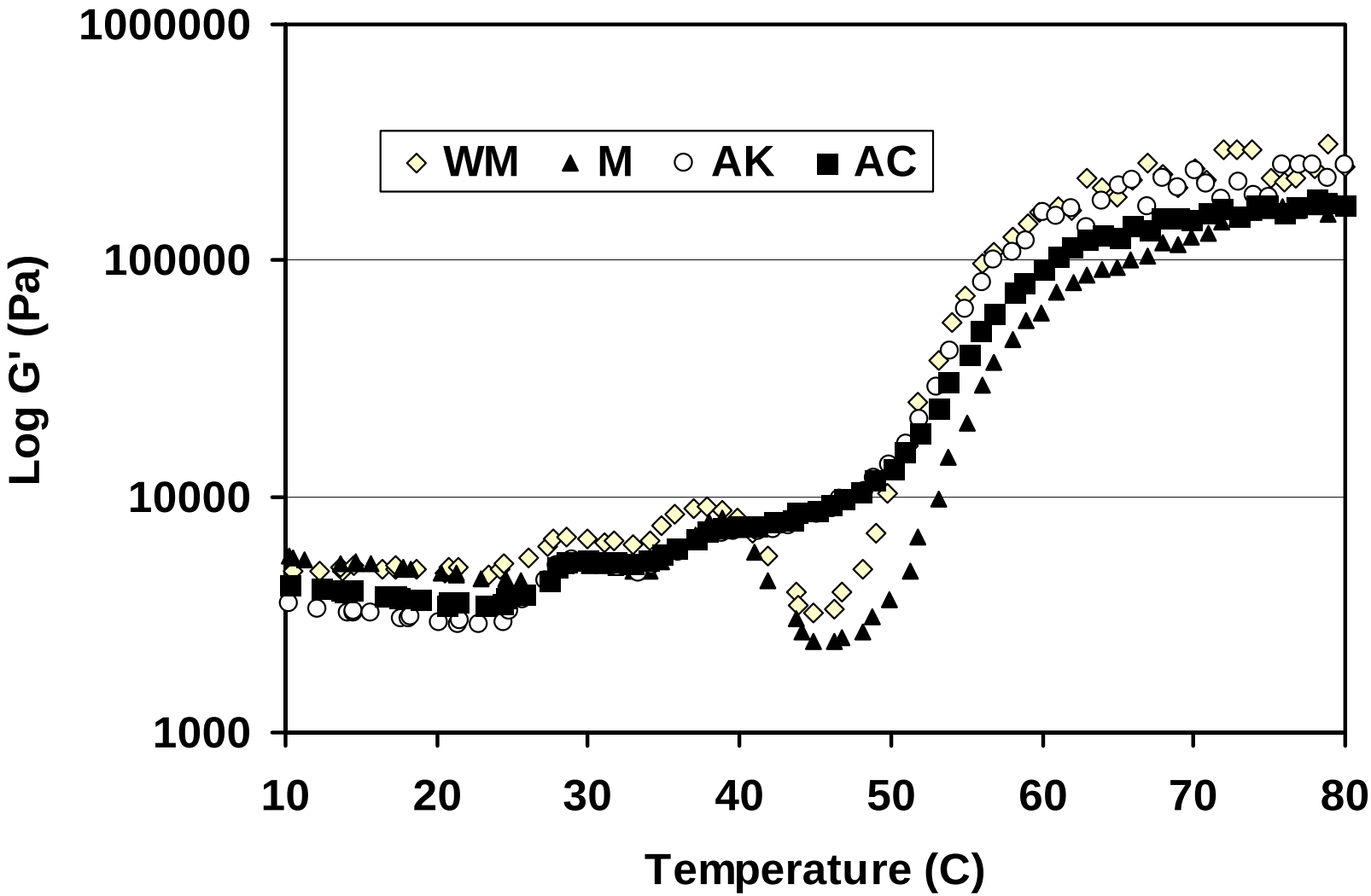
Superior Gelling Properties of FPI compared to Conventional Surimi

Fracture Analysis – Pacific Whiting



(Dr. Park's Lab, 2003)

Dynamic Gel Property – Rock Fish



(Yongsawatdigul and Park, 2004)

Texture of menhaden surimi and FPI

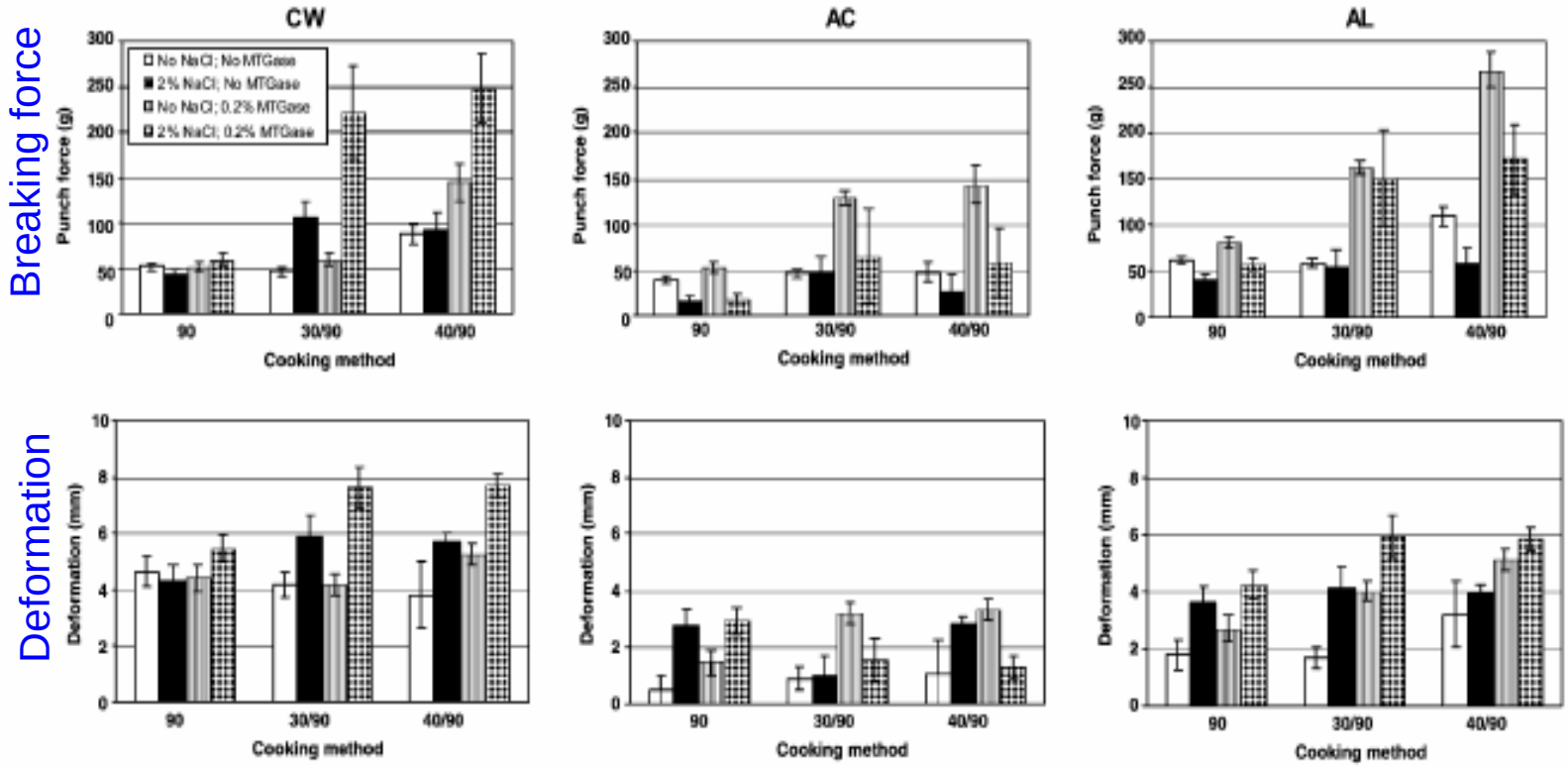
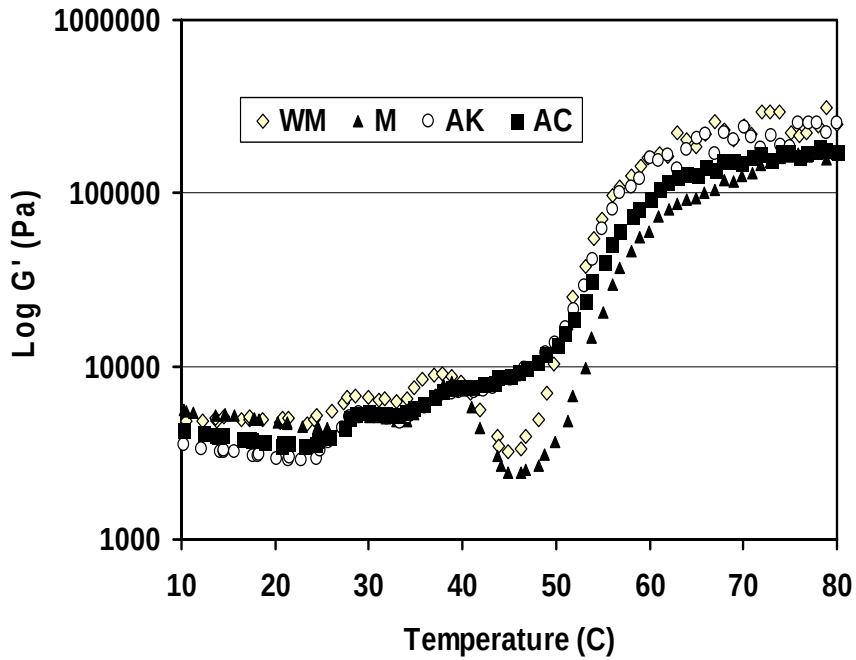


Fig. 1. Comparison of puncture test values for surimi gels made with three surimi types: conventionally washed (CW), acid-solubilized (AC) and alkaline-solubilized (AL). Gels were made with or without 2% NaCl, and with or without 0.2% added microbial transglutaminase (TGase). 90 indicates that gels were 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
Thawornchinsombut 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 ?

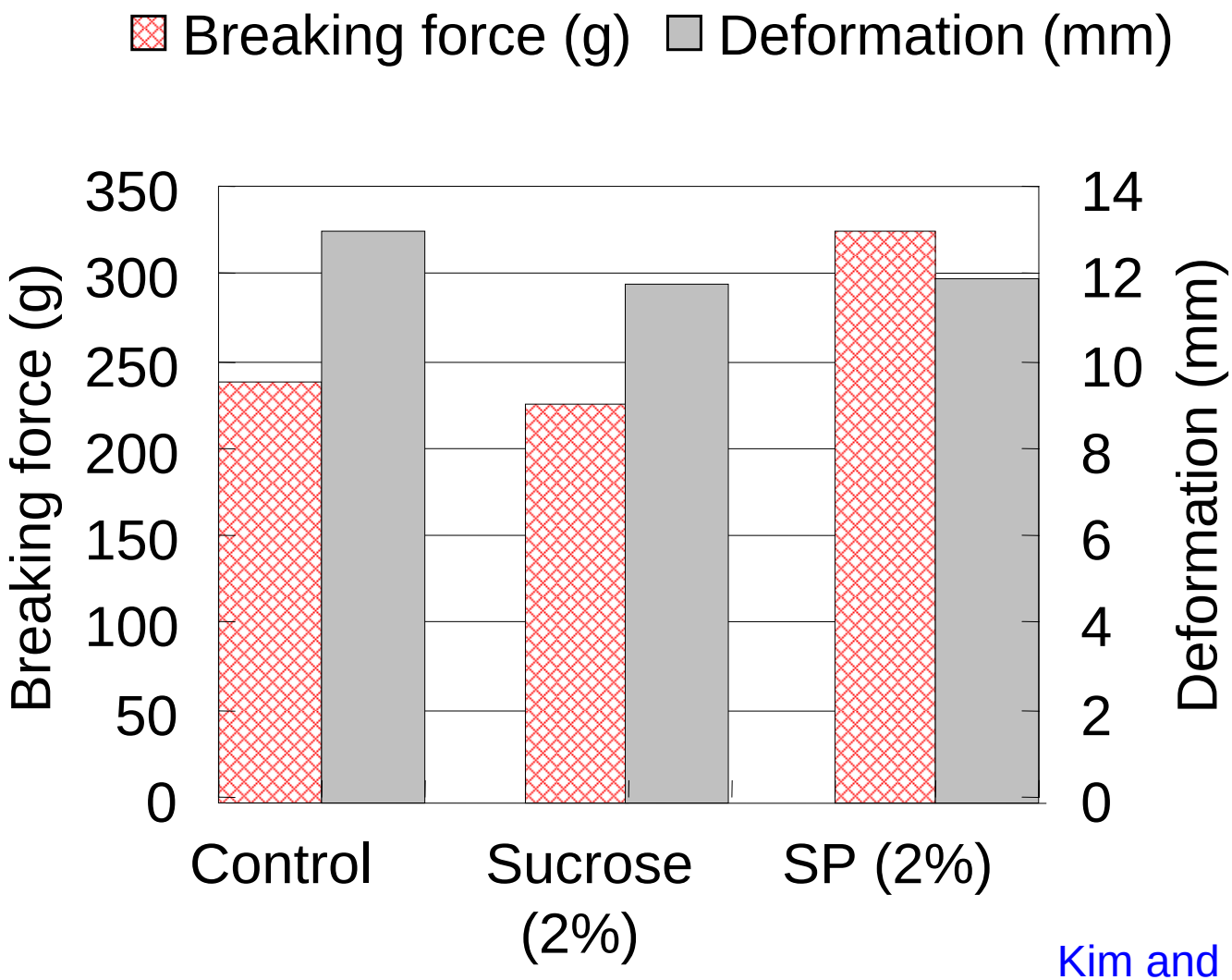
Superior Gelling Properties of FPI: WHY?

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



Kim and Park (2005)

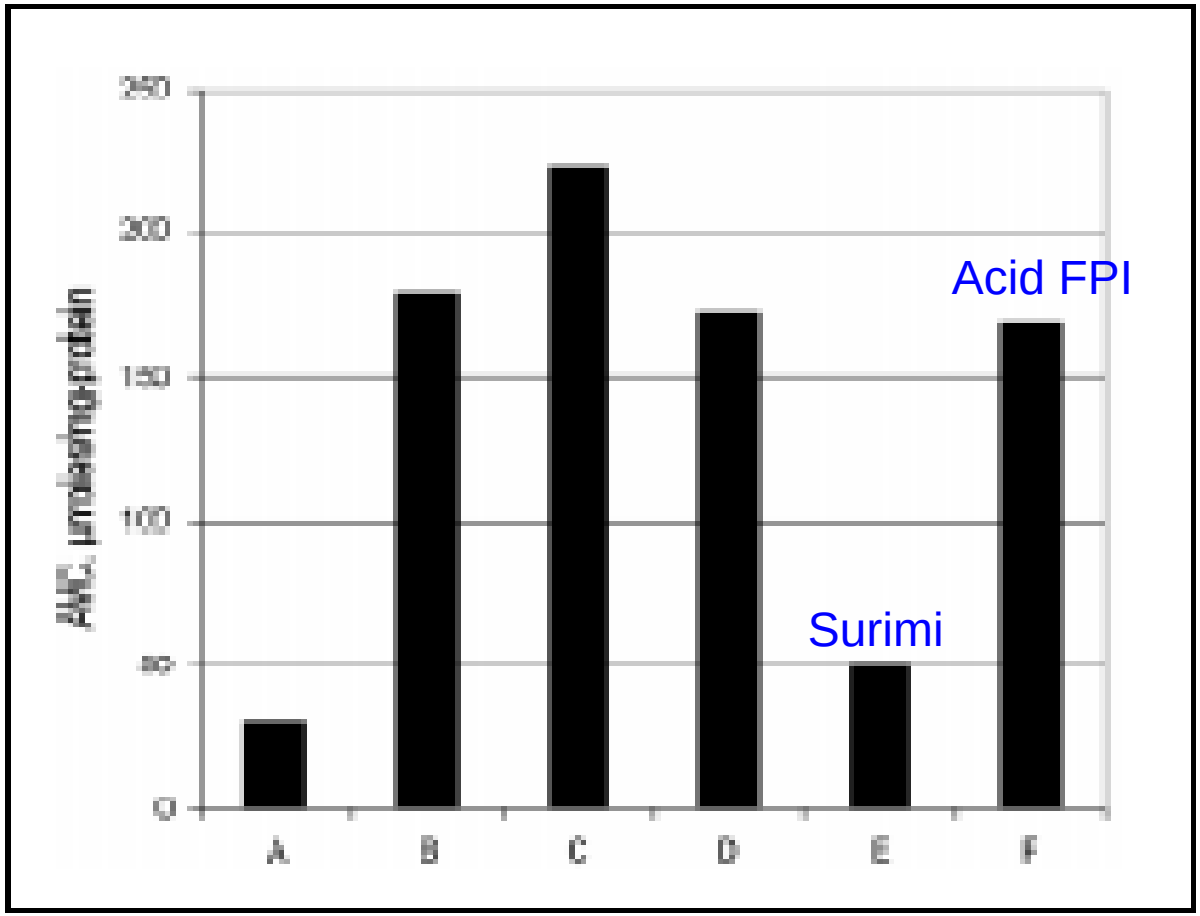


Figure 3—Cathepsin L activities of samples at various treatments: A: Water-soluble protein after 1-washing cycle; B: Water-soluble protein after 3-washing cycle; C: Supernatants after pH 2.5 treatment; D: Supernatants after pH 5.5 treatment; E: Surimi after 3-washing cycle; F: Surimi from acid-aided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

Cathepsin – L Activity

Pacific whiting

Choi and Park (2002)

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) Catfish

Sample	L^*	a^*	b^*	Whiteness
Surimi	70.4 ± 1.1a	−0.9 ± 0.2a	0.7 ± 0.4a	70.4a
Acid PI	73.8 ± 0.4b	−3.6 ± 0.2c	5.7 ± 0.3c	72.9b
Acid PI (skip 1st centrifugation)	75.1 ± 0.3c	−2.3 ± 0.2b	7.9 ± 0.5d	73.8c
Alkali PI	75.0 ± 0.7c	−3.0 ± 0.2 c	0.2 ± 0.4a	74.8d
Alkali PI (skip 1st centrifugation)	78.4 ± 0.3d	−2.2 ± 0.1b	3.2 ± 0.0b	78.1e

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

Kristinsson et al (2005)

Table 1—Color values of samples prepared by various treatments[a] Rockfish

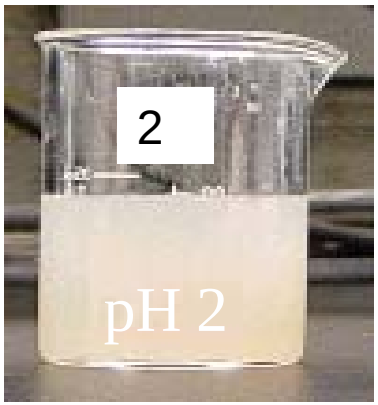
Sample	L^*	a^*	b^*	Whiteness ($L^* - 3b^*$)
M	77.63^{c}	2.60^{a}	7.19^{a}	56.07^{d}
WM	81.22^{a}	1.69^{b}	0.83^{d}	78.74^{a}
AC	79.06^{b}	1.17^{c}	4.74^{c}	64.84^{b}
AK	76.20^{d}	-0.04^{d}	5.67^{b}	59.18^{c}

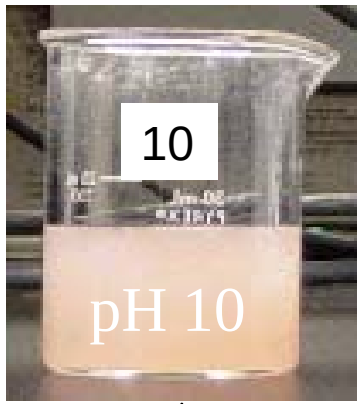
[a]Means with different letters in the same columns are significantly different ($P < 0.05$).

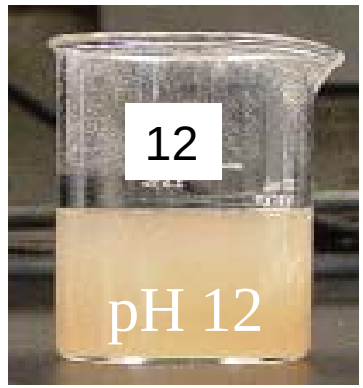
Yongsawatdigul and Park (2005)

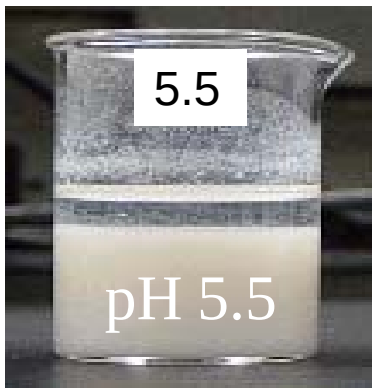
Effects of pH on the appearance of **Sarcoplasmic Proteins** (SP) from Pacific whiting

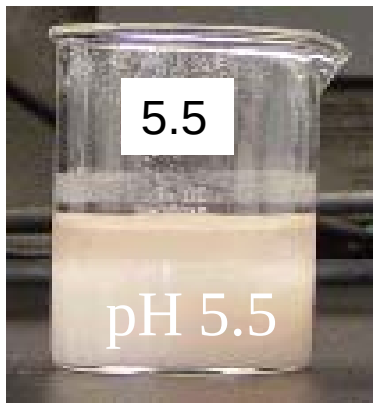
SP (pH 7)
(No NaCl)

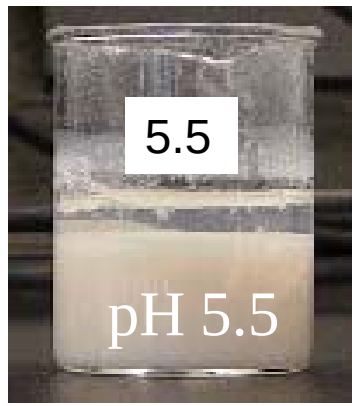




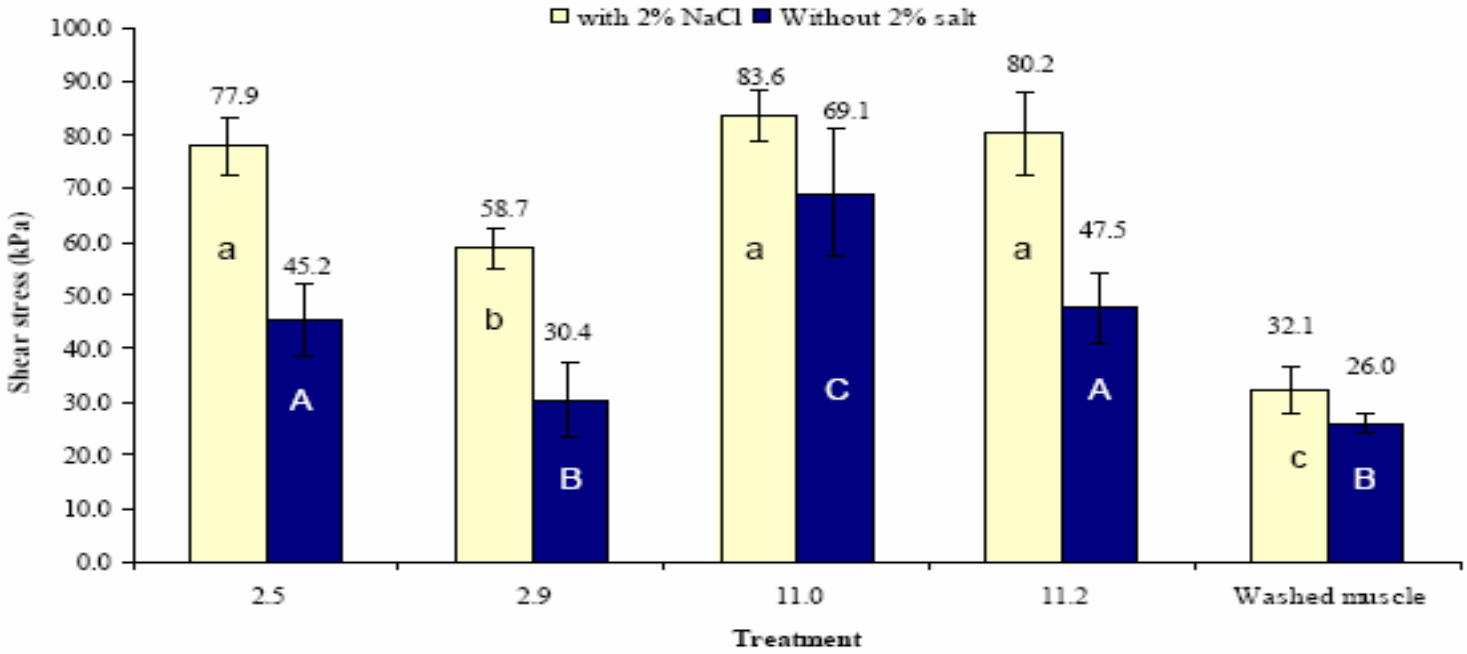








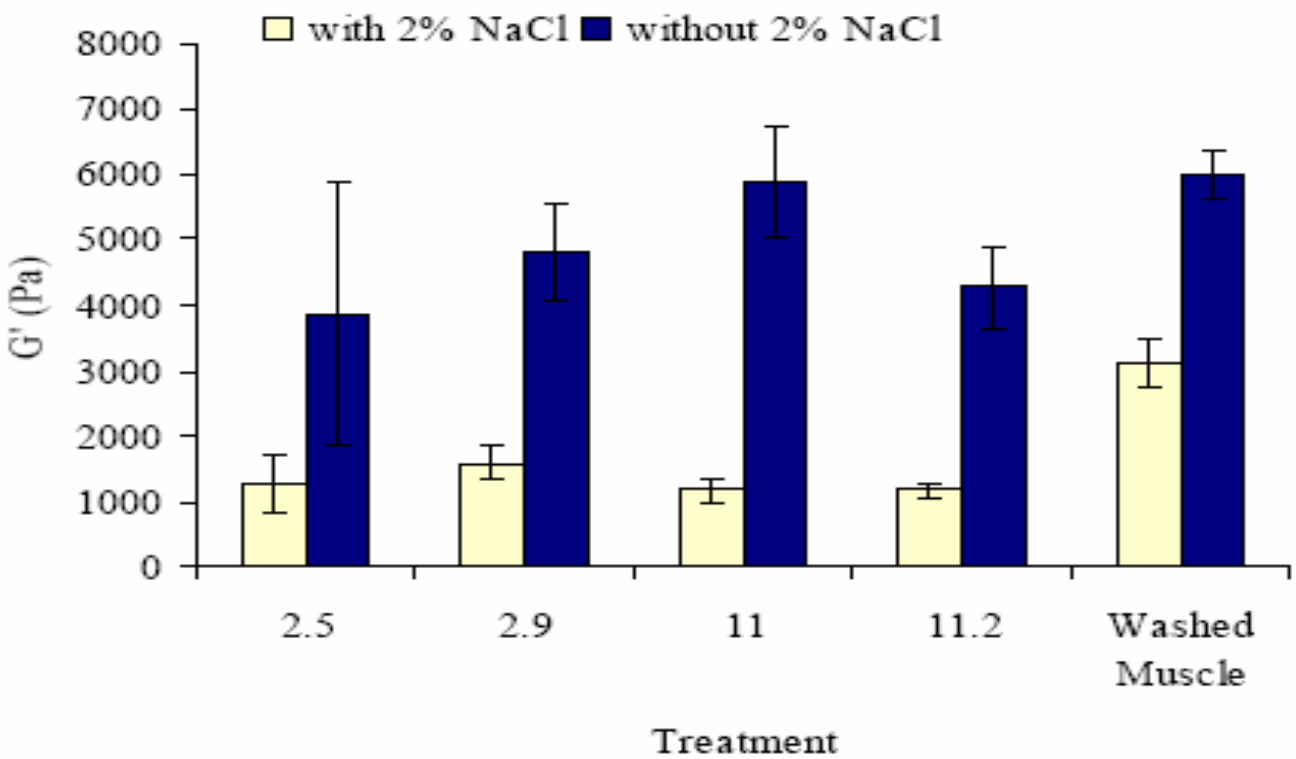
Effect of salt addition on gelation of FPI



Ingadottir (2004)

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 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

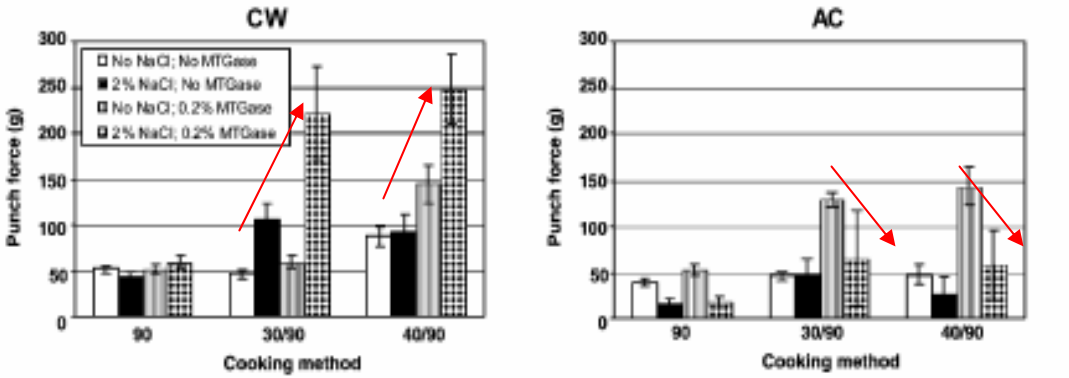


Ingadottir (2004)

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

Breaking force

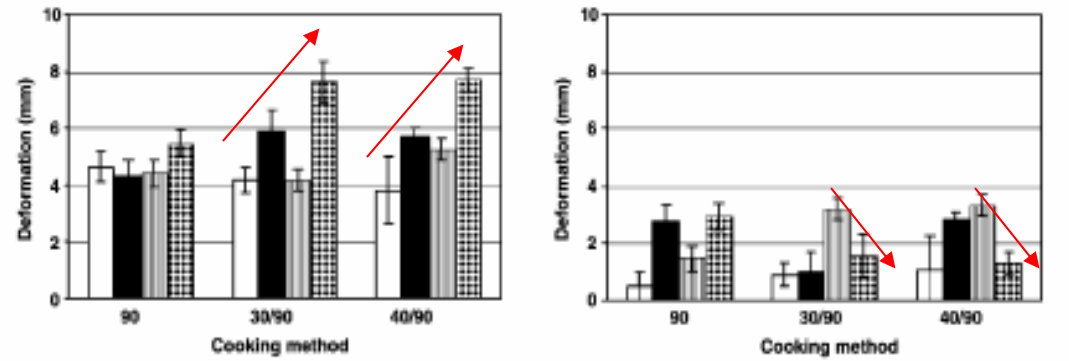


Salt addition on FPI:

Salt addition:

Negative on strength

Deformation



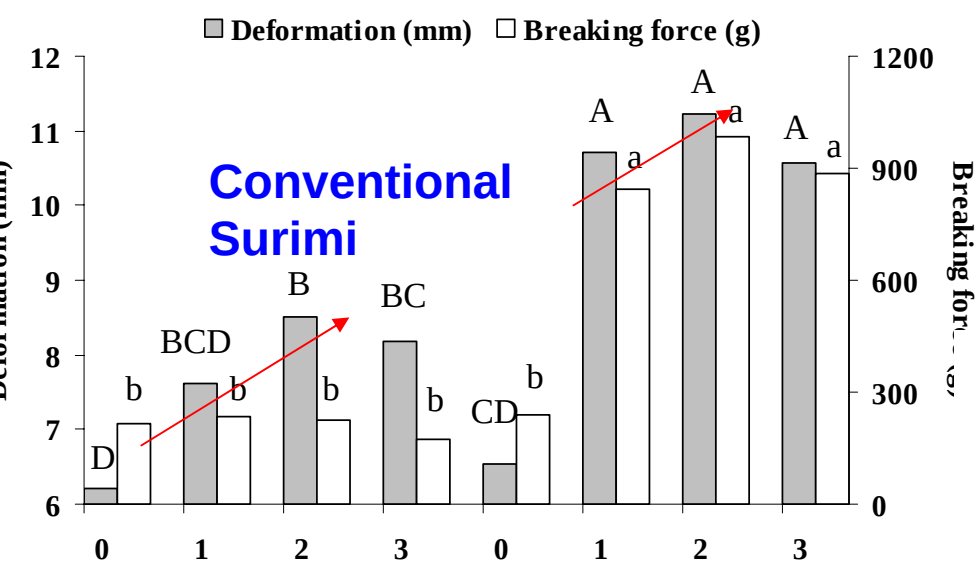
Salt addition:

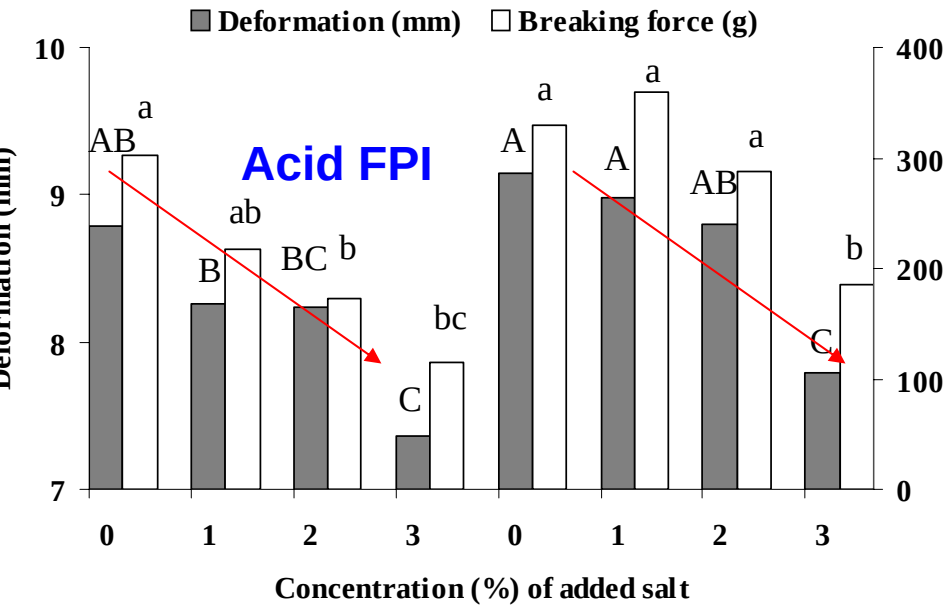
Negative or Positive on deformation

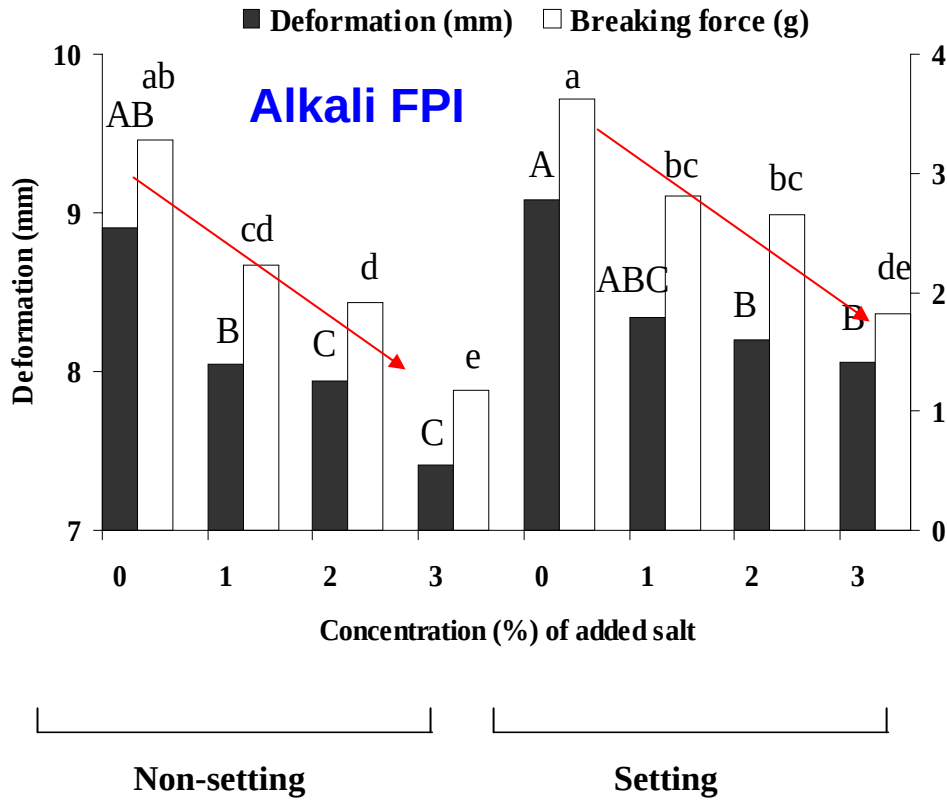
Fig. 1. Comparison of puncture test values for surimi gels made with three surimi types: conventionally washed (CW), acid-solubilized (AC) and alkaline-solubilized (AL). Gels were made with or without 2% NaCl, and with or without 0.2% added microbial transglutaminase (TGase). 90 indicates that gels were 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

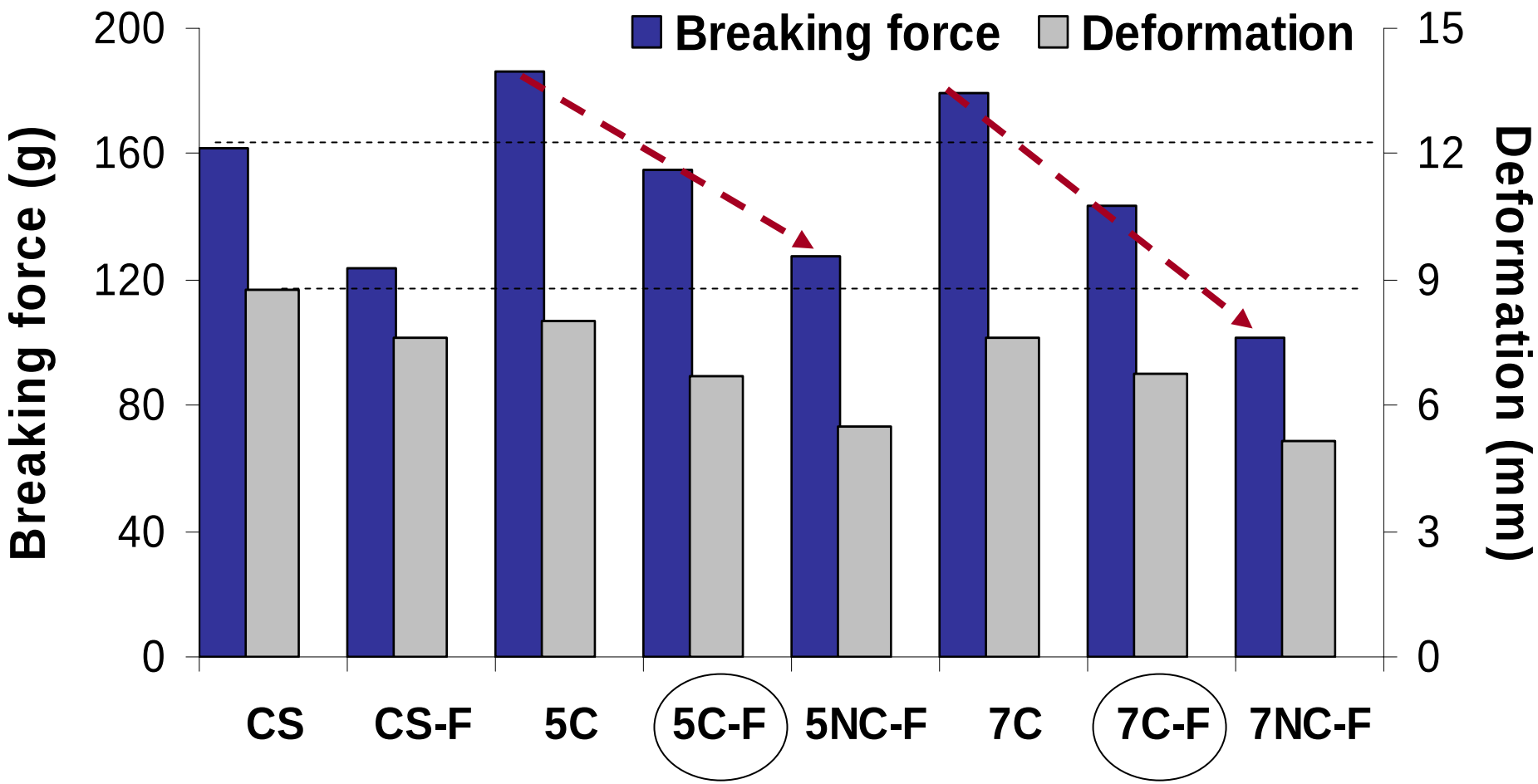






Kim and Park, 2008

Should FPI be stored frozen with cryoprotectants? At what pH?



(Thawornchinsombut and Park 2004)

Current Commercial Players

- **MPF Inc**
  - Alkaline and Acid solubilzation
  - 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%

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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 6.0 ~ 6.5

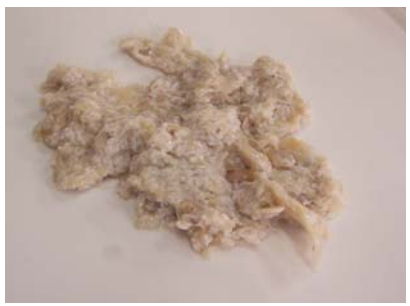
2 N NaOH

pH 11

2 N HCl

pH 5.5

pH 7.0



2 N NaOH

FPI

(Fish Protein Isolate)

$$NaOH + HCl = NaCl + H_2O$$

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?

$NaOH + HCl = NaCl + H_2O$

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…..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