

A Rapid, Easily Used Test Kit
To Determine Histamine Concentrations in Fish

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BACKGROUND

Recently the U.S. Food and Drug Administration announced it was improving its histamine policy in the revised Compliance Policy Guide 7108.24 Decomposition and Histamine “Raw, Frozen Tuna and Mahi-Mahi; **Canned** Tuna; and Related Species” (Fed. Reg. v. 601149, August 3, 1995; pp. 39754-39756). In summary the FDA:

- lowered the Defect Action Level (DAL) to 50 ppm for decomposition.
- eliminated the requirement that findings of < 200 ppm had to be confirmed by organoleptic tests [i.e., histamine determination alone is sufficient].
- application of the revised DAL applies to raw and frozen **tuna** and **mahi-mahi**; and furthermore, on a case-by-case basis histamine levels ≥ 50 ppm to < 500 ppm may be used as evidence of decomposition in other species.
- the Action Level (AL) of 500 ppm will apply to species of fish that have been implicated in histamine poisoning outbreaks.

The FDA notice calls our attention to the fact that “. . .**nonvolatile** spoilage compounds such as histamine remain in the product (the fish) and can be determined reliably by chemical analysis.” The most commonly used method for histamine determination is AOAC Official Method 977.13--the **fluorometric** method, which received Final Action approval in 1987. This method has **three** phases:

First - extraction of histamine **from** it matrix (fish). This phase consists of a methanol extraction of histamine from (blended) fish followed by heating (60 °C) and filtrations (Fig. 1).

Second - **purification** of the histamine **from** the “extract.” This phase involves ion exchange chromatography whereby histamine passes through the column but interferences, such as **histidine** and other free amino acids are retained.

Third - detection of histamine in the column effluent. This phase involves adjusting the sample to alkaline pH, reaction with OPT (o-phthalic dicarboxaldehyde a.k.a. OPA), sample neutralization followed by fluorescent detection ($\lambda_{ex} = 350 \text{ nm}$ and $\lambda_{em} = 444 \text{ nm}$) (Fig. 2).

Quantitative histamine concentrations are determined by comparing sample fluorescence values to a standard curve generated daily or more often. Standards are prepared in a fashion that they are subjected only to the detection phase of the method.

THE NEW “OLD” ASSAY

Our assay utilizes an AOAC approved chemical method (spectrophotometric) in a new, **user-friendly** format. The first phase, the extraction procedure is identical to the fluorometric AOAC method.

The second phase uses an anionic exchange resin to remove interfering substances only in a batch-wise fashion by means of a simple filtration device. The filtrate is **pH** adjusted by means of dilution and a $100 \mu\text{L}$ aliquot is added to a detector cup. Then, captured histamine is reacted with diazotized **p-nitroaniline** (previously activated by reacting sodium nitrite in a crushable ampoule within a tube of **p-nitroaniline**). The reflectance value of the reaction product (**diazo dye**), which is reddish colored, is measured in a simple reflectometer, the **AgriMeter** (Fig. 3, 4).

Table 1 shows the reflectance ranges for important levels of histamine including the Defect Action Level of 50 ppm.

Figure 5 shows a standard curve with error bars derived from values **like** those in Table 1.

Figure 6 is a “scattergram” comparing the fluorometric AOAC method values for 85 samples of canned and **fresh frozen** tuna to the ranges of the **Alert™** for Histamine test with these same samples. Please note the single high sample (ca. 53 ppm) in the $> 5 < 19$ range. Upon examination, that particular sample was found to have a high salt content.

Figure 7 shows the effects of salt on the Alert assay; i.e. at $> 2.0 \%$ (w/w) salt a standard curve is changed markedly. However, canned tuna should have $\leq 1.0\%$ salt, a specification that is determined separately in Good Manufacturing Practices (QA) procedures.

Table 2 lists histamine levels in nine samples of **fresh/frozen mahi-mahi** as determined by the AOAC fluorometric method and the Alert for Histamine test both of which used standard curves. The classification column was the conclusion drawn by the technologist conducting the test. It should be noted that extractions with 75% methanol were used.

ACKNOWLEDGMENTS

We acknowledge with thanks the interests of Drs. Walter Staruszkiewicz, Patricia Rogers and George Hoskin of the FDA's Office of Seafood. In addition, we would like to acknowledge the support of the tuna canning industry which supplied samples of fresh/frozen and canned tuna for the evaluation of the Alert for Histamine test.

Fig. 1

AOAC Method of Extraction

Homogenize Sample (AOAC 937.07)

↓

Extract 10 g in
50 ml 100% MeOH in
Blender 2 min.

↓

Heat Sample to 60°C
in H₂O Bath, Incubate
15 Min.

|

Cool to 25° and
Dilute to 100 ml

|

Filter Through Folded
Filter Paper

|

Sample Now Ready
For Purification

Fig. 2

AOAC Fluorometric Method

Purification

Add 1 ml Extract
to 8 cm Prepared
Dower Column*

↓

Immediately Initiate
Flow By Adding 5 ml H₂O

↓

Add Proportionately Larger
Volumes of H₂O into Flask
Containing 5 ml 1 N HCl Until
~ 35 ml Has Been Eluted

↓

Dilute to 50 ml

↓

50 ml Sample Now Ready
For Assay

Assay

Add 5 ml Eluted
Sample to 50 ml Flask

↓

Add 10 ml 0.1 N HCl

↓

Add 3 ml 1 N NaOH

↓

Within 5 Min. Add 1 ml
(0.1%) OPA and Incubate 4 Min.

↓

Add 3 ml 3.57 N Phosphoric Acid

↓

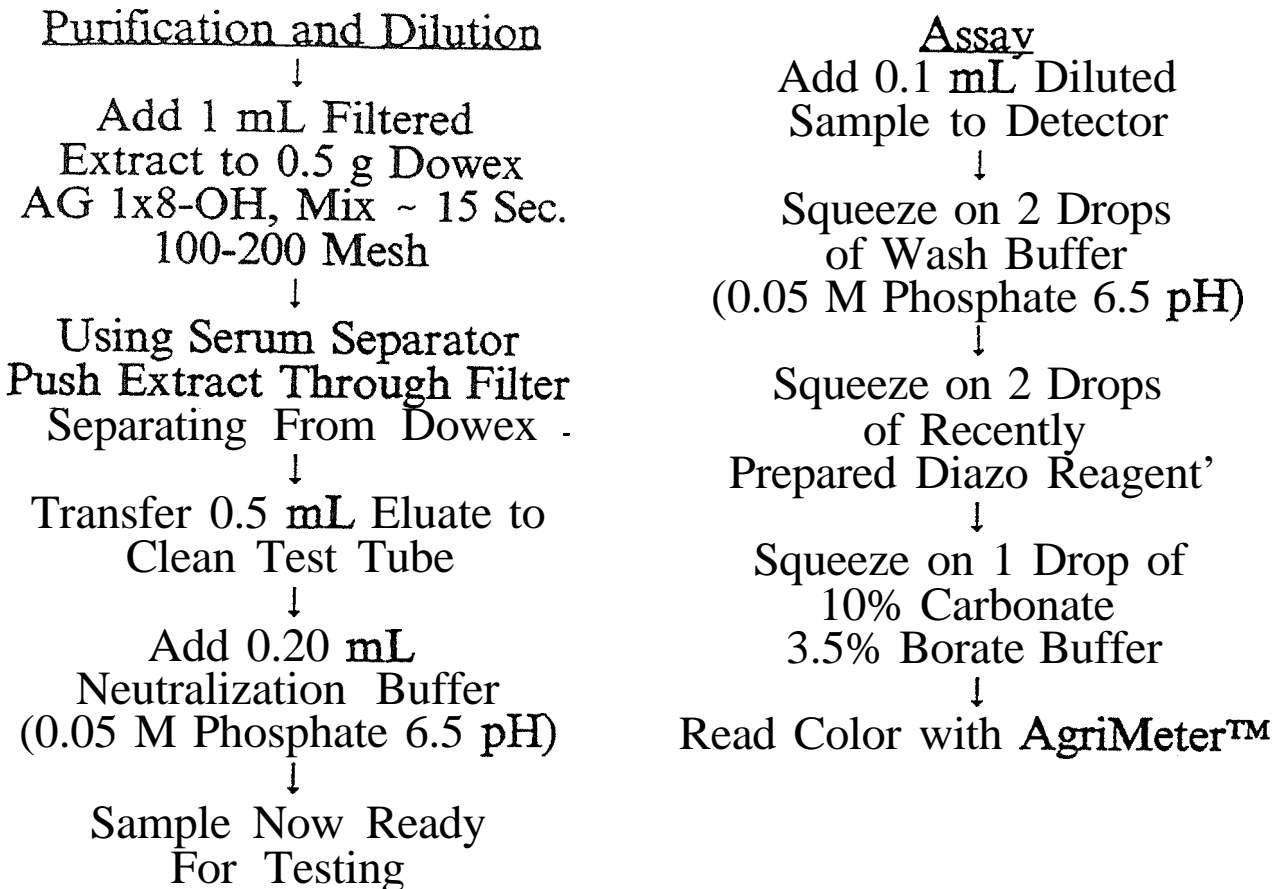
Within 1.5 Hr. Read Fluorescence
Ex 350 nm Em 444 nm

*Prepare columns before test by (preparing weekly):

1. treating with 1N NaOH for approximately 1 hour (15 ml/g resin);
2. washing extensively;
3. transferring resin to columns 8 cm in height;
4. washing column with 10 ml of H₂O prior to purification.

Fig. 3

ALERT® HISTAMINE ASSAY



¹The diazo reagent is made by reacting p-nitroaniline (0.1 g/100 mL 0.1 N HCl) with NaNO₂ (4 g/100 mL H₂O). This is done in a dropper bottle by crushing a glass ampoule containing the NaNO₂; allowing it to mix with the solution of p-nitroaniline. This activated reagent is immediately ready for use and is stable for up to 8 hours.

Fig. 4

HISTAMINE CAPTURE AND DETECTION

p-nitroaniline in HCl

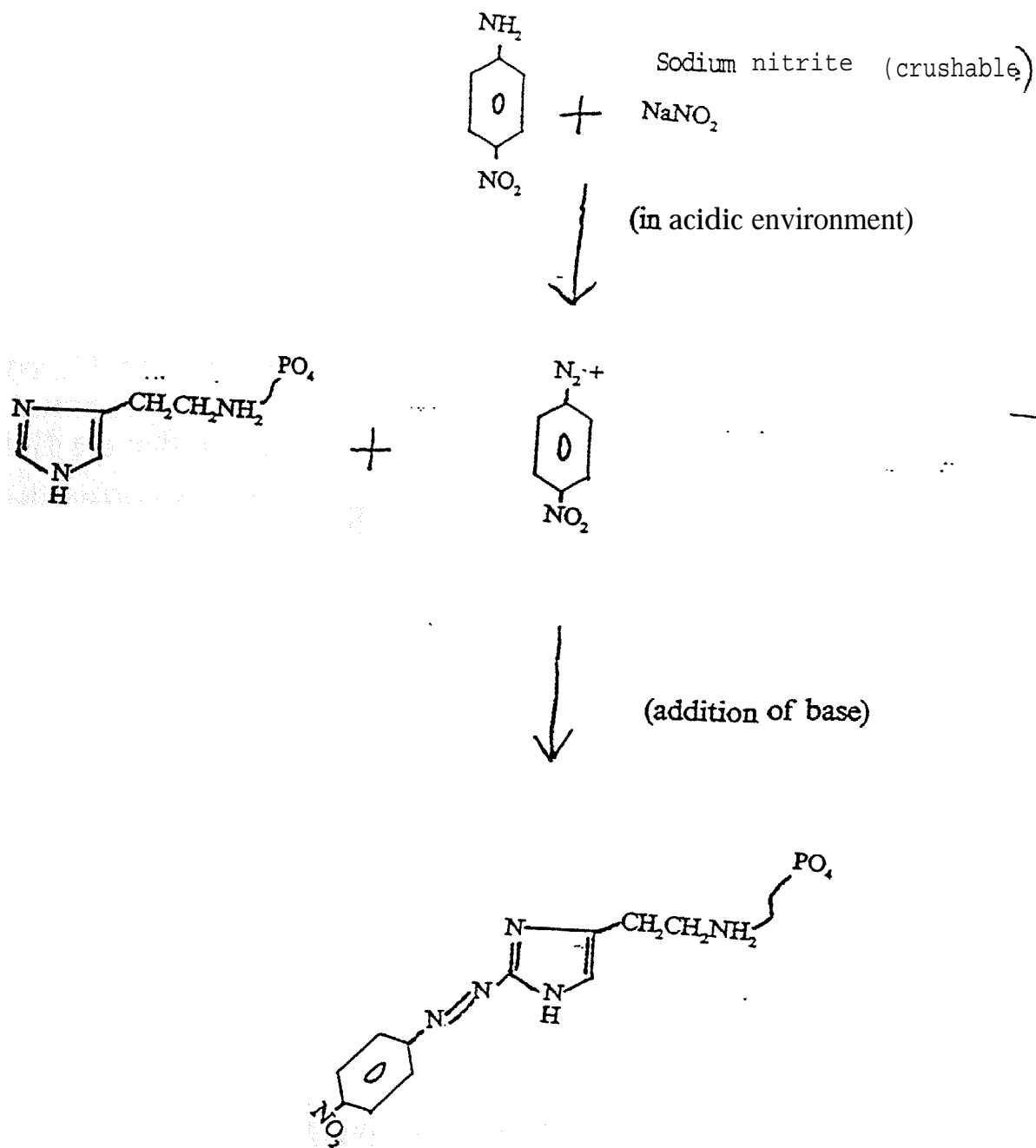


Table 1

Critical Control Levels of the Alert[®] for Histamine Test

<u>Histamine Level ($\mu\text{g}/\text{mL}$)</u>	<u>Reflectance (Arbitrary Units)</u>	
	<u>$X \pm 2 \text{ S.D.}^1$</u>	<u>Range</u>
0	515 \pm 40	475 - 555
5	465 \pm 40	425 - 505
20	365 \pm 20	345 - 385
50	225 \pm 35	190 - 260

¹Critical control levels were established using 10 runs/meter and 3 meters ($n = 30$).

Critical Control Levels of the Alert® for Histamine Test

Fig. 5

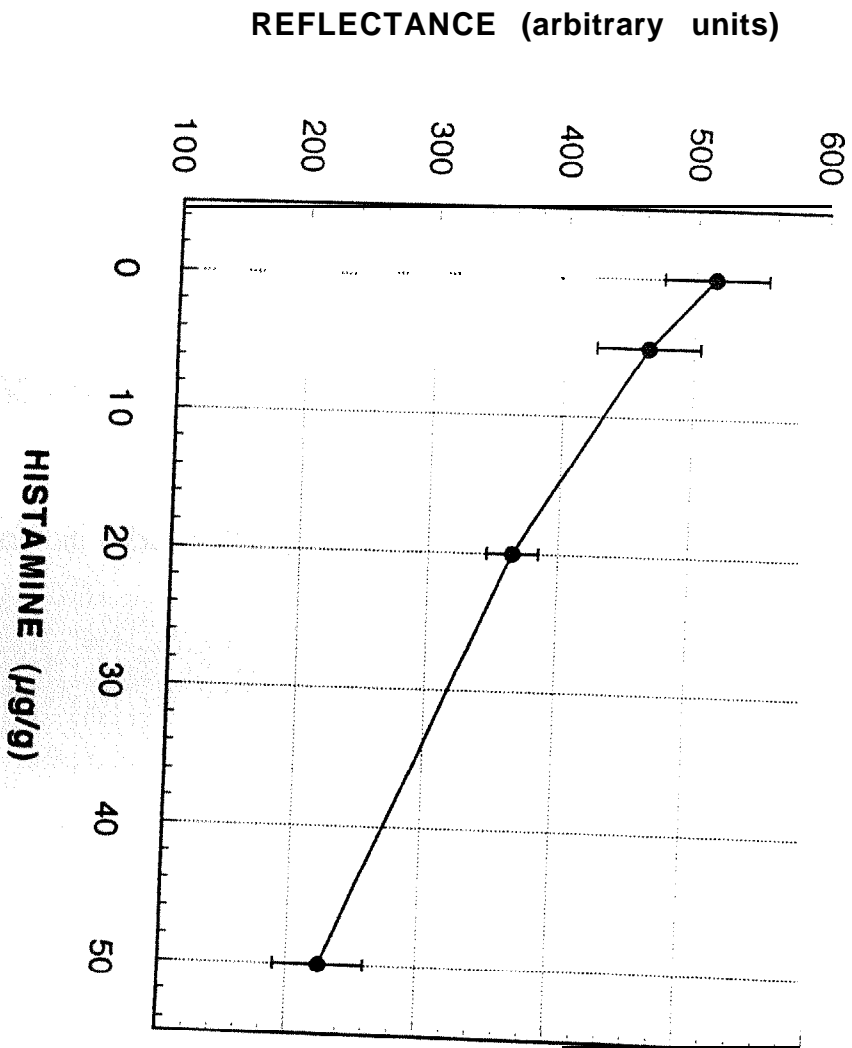
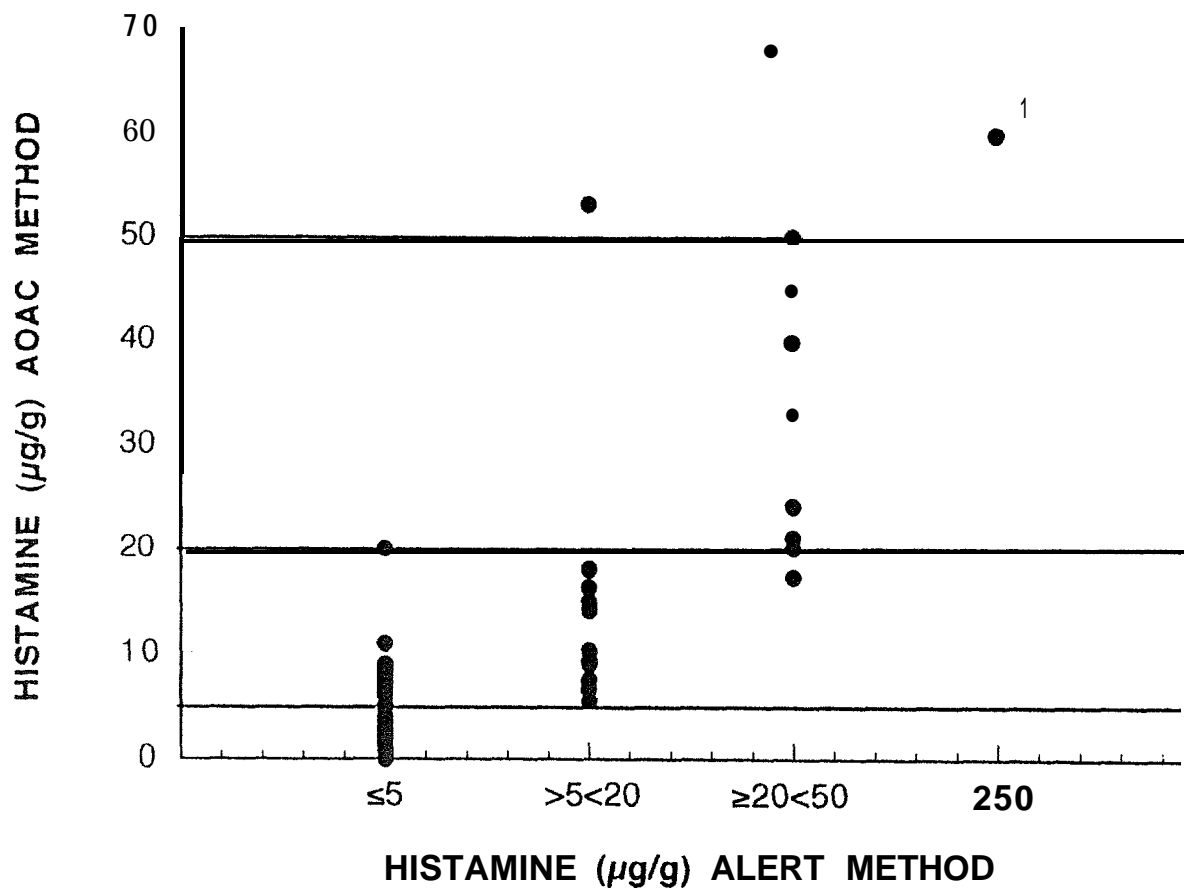


Fig. 6

**Comparison of the Alert for Histamine Test
and the AOAC Fluorometric Method
in Canned and Fresh Tuna**



Seven additional samples fell into this range that exceeded 70 µg/g.

- 0 % (w/w) salt
- 0.25 % (w/w) salt
- △— 0.50 % (w/w) salt
- ▽— 1.0% (w/w) salt
- ◇— 2.0% (w/w) salt
- ⊙— 4.0% (w/w) salt

Fig. 7

Salt Effects in the Alert^R for Histamine Test

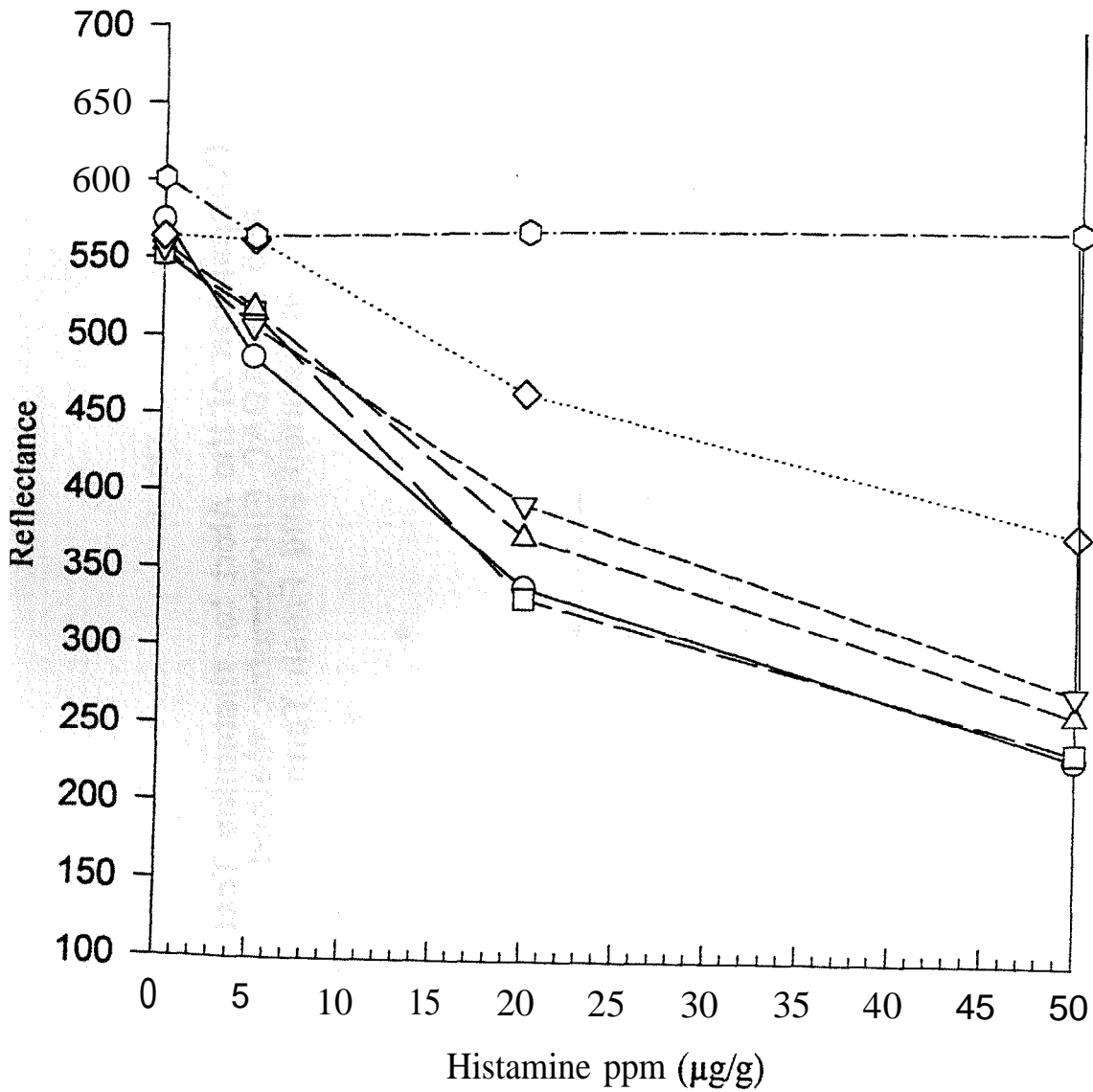


Table 2

**Detection of Histamine Levels in Mahi Mahi using AOAC
Fluorometric Method and the Alert[®] for Histamine Test:
Prepared by FDA Office of Seafood**

Fish #¹	AOAC Fluorometric Method	Alert for Histamine²	Classification
1	24	37	P
6	106	> 50	F
7	1466	Hi (> 750)	Toxic
10	2	5	P
14	1138	Hi	Toxic
16	10	13	P
17	191	> 50	F
22	162	81	F
22-2	148	175	F

¹Extracted in 75% MeOH.

²Levels determined using a standard curve in the Alert for Histamine Format.

IMMUNOLOGIC APPROACHES TO THE IDENTIFICATION OF FISH PROTEINS:
TOWARD SPECIES-SPECIFIC "FIELD KITS"
FOR STUDIES IN ECOLOGY AND SEAFOOD TECHNOLOGY

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Introduction The use of immunological methods to investigate basic problems in oceanographic research and marine or freshwater ecology provides sensitive and specific tools with wide versatility and applicability (I, 11,12). Polyclonal and **monoclonal** antibody probes can be labeled with radioisotopes, **fluorochromes**, enzymes, metals such as colloidal gold, and by **biotin-avidin** technology for **immunoassays**; the probes are detected microscopically, with automatic flow **cytometry** devices, by electrophoretic methods, scintillation counters, and automated microplate readers (6). Although formerly used almost exclusively in clinical and basic medical research, increasing numbers of investigators are adapting these methods for studies in both applied and theoretical marine biology, and **also** seafood **technology**.

In this report we have used immunological techniques to investigate predator-prey relationships with recently released red drum fingerlings (*Sciaenops ocellatus*, Linnaeus), and to distinguish early life history specimens of specific species of the family Lutjanidae. The **immunological** detection methods described here are also applicable for problems associated with **identification** of commercial seafood products and safety, and are valuable for preventing deliberate or **unintentional** substitution of inferior products for valuable fish species (4).

Immunologic methods to assess predation of hatchery-reared juvenile red drum (*S. ocellatus*).

During the last seven years, the University of Miami has been part of a cooperative stock **enhancement** program with Florida International University (Miami, FL), and the Florida **Department** of Environmental Protection. The purpose of the program has been to test the feasibility

of reestablishing and enhancing red drum populations in Biscayne Bay, Florida, because of the virtual disappearance of the species from coastal waters in this area. Since 1988, hundreds of thousands of hatchery-reared red drum fingerlings have been released at mainly two sites, Rickenbacker Causeway (Hobie Beach) in north-central Biscayne Bay, and Matheson Hammock, located on the mangrove-lined western shore of central Biscayne Bay⁴.

Our role in the red drum stock enhancement program has been to investigate predation of newly released fingerlings. The program appeared to be ideal for predation studies because after each release, the red drum fingerlings remained inshore in shallow aquatic vegetation close to the release site for at least 10 hours, facilitating the capture with a center-bag seine of a number of potential predators attracted to the site.

Visual examination limited the identification of many of the specimens found in gut contents of the two major predators seined at both of the release sites, *Sphyraena barracuda* (Walbaum) (great barracuda) and *Strongylura notata* (Poey) (redfin needlefish). Many of the ingested fish were partially proteolysed and could not be identified as to the species. Therefore, we have used immunological methods to develop reagents for assays to detect red drum in the gut contents of predators.

Initially, a goat polyvalent antiserum was produced to soluble red drum whole fish extract (anti-RDE serum) for use as the primary antiserum in Western blots. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with soluble extracts of 12 different species of fish found in Biscayne Bay (Table 1), followed by Western blots with the anti-RDE serum. A red drum 80 kDa protein was selected that was not found in soluble extracts of the other fish. The protein was purified to homogeneity by sequential steps of solid ammonium sulfate fractionation and anionic and cationic exchange resins using the Fast Protein Liquid Chromatography system (FPLC) (11). The highly purified 80 kDa protein was a single chain polypeptide that reacted positively with periodic acid-Schiff reagent, indicating a glycoprotein. It was found in soluble extracts as two isoforms in small fish (*ca.* 1.5 cm.), but as a single protein from fish greater than 17 cm in length. The amino terminus of the 80 kDa protein was not blocked, and structural information is shown in Table 2. Further structural work for the purpose of developing assays of the protein (e.g., ELISA) was not carried out at this time. A goat antiserum was produced (anti-80 kDa gp) which was used as the primary antiserum in Western blots to test red drum fingerling extracts. Although both preimmune and immune goat sera reacted nonspecifically with extracts of many of the fish (e.g., silver jenny), adsorption of the antiserum with glutaraldehyde-insolubilized silver jenny extract resulted in an antiserum reactive only with the 80 kDa red drum protein and not with any proteins from the 12 species of fish and three invertebrate species selected for these studies (11).

⁴Rickenbacker Causeway (Hobie Beach): [25°44'50" (Lat.); 80°13'20" (Long.)]; Matheson Hammock: [25°44'15" (Lat.); 80°15'20" (Long.)].

Table 1. Fish collected in Biscayne Bay, Florida, and tested with goat anti-red drum (*Sciaenops ocellatus*) extract serum. Soluble extracts of each fish did not contain a 80 kDa protein found in soluble extracts of red drum.

Name	Rank in Abundance*
Silver jenny (<i>Eucinostomus gula</i>)	1
Gulf toad fish (<i>Opsanus beta</i>)	2
Bluestriped grun (<i>Haemulon sciurus</i>)	4
Gray snapper (<i>Lutjanus griseus</i>)	5
White grunt (<i>Haemulon plumieri</i>)	6
Fringe filefish (<i>Monacanthus ciliatus</i>)	8
Tomtate (<i>Haemulon aurolineatum</i>)	14
Bronze cardinal fish (<i>Astrapogon alutus</i>)	16
Silver perch (<i>Bairdiella ch rysoura</i>)	20
Sea trout (<i>Cynoscion nebulosus</i>)	30
Spotfin mojarra (<i>Eucinostomus argenteus</i>)	not listed
Bar jack (<i>Caranx ruber</i>)	not listed

*RSMAS, rollerframe trawl, July-December, 1993, Biscayne Bay, Florida.

Table 2. The N-terminal sequence analysis of the red drum 80 kDa glycoprotein*.

Ala Pro Ala Asn Lys Val Glu/Lys Lys Ala Val/Pro Lys/Glu Ser Asn Gln Glu His Gln Lys Lys
 Met Asp Leu Ala Ala Lys

*Analyzed at the University of Florida, Dept. of Biochem & Mol. Biol. (ICBR Protein Chemistry, Core Laboratory), Gainesville, FL.

Red drum fingerlings were fed to both adult sea trout and bar jack in controlled laboratory feeding experiments. The red drum protein was detected in stomach contents by Western blots after 1 and 2 h, but was not found after 4 h using the anti-80 kDa gp IgG fraction as the primary antibody. Three breakdown products of the 80 kDa protein were detected in prey after 2 h using Western blots: 58, 37, and 28 kDa. In order to facilitate testing multiple samples of red drum soluble extracts, we also developed a highly sensitive and specific Enzyme-linked Immunosorbent Assay (ELISA) for the 80 kDa protein for an automated microplate reader (1).

Fish sampling was started the morning after the release of batches of several thousand red drum fingerlings into the sites on Biscayne Bay. Both newly released red drum fingerlings and indigenous predators were captured by seining and the predators were immediately frozen with dry ice to prevent proteolysis of engulfed fish. Visual examination of the guts of *S. barracuda* and *S. notata* in the laboratory showed both identifiable and unidentifiable fish remains. Soluble extracts of the latter were tested in indirect ELISA, and from standardized linear plots of the average O.D.₄₉₀ versus the 80 kDa red drum protein concentration, it was possible to detect ng quantities of the 80 kDa red drum protein in the fish extracts (1). Thus, it was possible to detect unrecognizable red drum fingerlings in gut contents of predators in nature by both Western blots and ELISA. Positive identification of the 80 kDa protein depended on the degree of decomposition in the gut, which was influenced by such factors as the species of predator, its feeding habits and metabolic rate, and the amount of time elapsed between ingestion of prey and capture of the predator. Since the predators are poikilothermic, the water temperature is also important for digestion rates.

Immunologic methods to assess early life history specimens of Family Lutjanidae.

The second major project is to produce immunologic reagents for the specific identification of eggs and early life history specimens of western Atlantic snappers (Family Lutjanidae). Specific species identification of early life history stages (especially eggs and young larvae) and the source of recruits of lutjanids are important research pursuits because 1) early life history stages are known for only a few species in their complex recruitment strategies, 2) overfishing occurs for several species, 3) habitat loss occurs because of pollution and development, and 4) they are of high commercial and recreational importance (8). Overfishing and habitat loss contribute to the unbalance of the fragile equilibrium of the reef community, and lutjanids constitute one of the major predators of coral grazing species. The innovative use of immunological methods may provide answers to identification problems for early life stages. These stages must be identified to species in order to understand recruitment mechanisms which determine adult population sizes. Currently eggs and early larvae cannot be identified because of morphological similarities. Only biochemical/immunological techniques offer promise to solve identification problems.

Based on unpublished data comparing the proteins in soluble extracts of different lutjanid species in SDS-PAGE in our laboratory, it may be possible to isolate a few species-specific proteins to produce polyclonal antisera for identification of individual life history stages of *Lutjanidae*. For example, soluble extracts of *Lutjanus griseus* (Linnaeus) contain a 66 kDa protein not detected in *L. vivanus* (Cuvier), *L. mahogoni* (Cuvier), *L. cyanopterus* (Cuvier), *Etelis oculatus* (Valenciennes) and a hybrid of *O. chrysurus* x *L. synagris* = *L. ambiguus*. In addition, *L. apodus* (Walbaum) soluble extracts contain at least one protein with a molecular weight greater than 66 kDa and two proteins less than 66 kDa that are not detected in *L. griseus*. Individual antisera to these proteins could be incorporated into portable, field-usable kits, such as a dotblot assay (3). However, there are 18 species of lutjanids in the western Atlantic distributed within five genera (9). The necessary time and costs would be prohibitive for purifying specific proteins from each of the 14 lutjanid species and one hybrid that are currently available to us, and for the animals required for immunization, antibody production, and testing.

The approach we have taken is to select a protein from SDS-PAGE gel profiles of soluble extracts of *L. griseus*. A 66 kDa protein was purified by FPLC utilizing successive steps of solid ammonium sulfate fractionation, cationic and anionic resins, and a molecular sieve gel. The highly purified *L. griseus* protein was injected into a adult female goat to produce a polyclonal antiserum that reacted with not only soluble extracts of *L. griseus*, but also reacted strongly with soluble extracts of *L. apodus* and *L. jocu* (Schneider). Additionally, the antiserum reacted weakly with soluble extracts of *L. buccanella* (Cuvier), *O. chrysurus* (Bloch), *Pristipomoides aquilonaris* (Goode & Bean), *L. synagris* (Linnaeus), *L. analis* (Cuvier), *Apsilus dentatus* (Guichenot), and *L. campechanus* (Poey) in Western blots. The anti-66 kDa antiserum reacted strongly with soluble extracts of oocytes, juveniles, and adults of *L. griseus* in Western blots. Most experiments have been with the 66 kDa protein of *L. griseus*, *L. apodus*, and *L. jocu*. In spite of the fact that the anti-66 kDa antiserum reacted strongly with the protein from the three lutjanid species in Western blots, evidence was obtained that each protein had both interspecies and species-specific determinants. For example, adsorption of the IgG fraction of the anti-66 kDa antiserum with glutaraldehyde-insolubilized *L. apodus* extract resulted in an antiserum that remained strongly reactive with *L. griseus* extracts but was weakly reactive with *L. apodus*, and negative with *L. jocu* in Western blots. In addition, the *L. griseus* protein was slightly heavier than the *L. apodus* and *L. jocu* proteins in SDS-PAGE and Western blots, although collectively the proteins from the three species are referred to as 66 kDa proteins. This information indicated that it will be possible to produce monoclonal antibodies using the 66 kDa protein, and the resulting antisera should selectively react with the three different species. Monoclonal antibodies have been used to identify a variety of other marine species (7, 5, 2). The next step is to produce immunofluorescent and immunoblot assays in order to identify specifically the eggs of each species in the plankton. Currently the 66 kDa protein from *L. griseus*, *L. apodus*, and *L. jocu* has been purified separately to homogeneity for comparative studies of the three proteins after amino acid sequencing. This will confirm the feasibility of monoclonal antibodies which are reactive with these three lutjanid species.

A dot-blot assay with the specific monoclonal antibody entails preparation of a diluted solution or suspension of a mixture of the life cycle form(s) of the lutjanid of interest. This is “dotted” onto nitrocellulose paper and incubated first with the specific mouse monoclonal antibody and second with a peroxidase-conjugated second antibody directed against the first mouse antibody. The peroxidase activity is assayed with H₂O₂ and 4-chloro-1-naphthol color development reagent. A positive reaction is visualized as a colored dot against the white nitrocellulose paper, and a negative reaction is colorless. The protein-dotted filter may be stored dry for several weeks without any loss of activity. Thus, specimens collected from many different sites could be stored and screened at the same time. Positive reactions are confirmed later in the laboratory on field-collected specimens using immunofluorescence in which the primary antibody is conjugated with a fluorochrome such as fluorescein and the bound antibody is visualized with a fluorescence microscope. A second procedure is to further test the diluted solution or suspension in an ELISA (1). Sarver *et al.* (10) has used the polymerase chain reaction to amplify portions of two mitochondrial genes to examine phylogenetic relationships of species of the Family Lutjanidae, but the relationships of some of the members are still unclear.

In summary, immunological methods were employed for two major research projects currently under investigation in our laboratories: 1) predation of hatchery-reared, juvenile red drum (*S. ocellatus*) following a program of reestablishing and enhancing the populations in Biscayne Bay, Florida, and 2) specific species identification of early life history stages (eggs and larvae) and the source of recruits of western Atlantic snappers (family Lutjanidae). After the development of immunoassays for these studies, the specificity and sensitivity of the methods have resulted in significant progress.

Immunological detection methods have also been valuable for many aspects of seafood science. Recently, Huang *et al.* (4) distinguished the commercially valued red snapper (*L. campechanus*) from less valuable substitutes using ELISA with two monoclonal antibodies raised to a red snapper protein. To ensure quality and safety of seafood products, immunology offers powerful methods for these goals.

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References

1. Arnold, P.I., Serafy, J.E., Clarke, M.E., *et al.* 1995. An immunological study of predation on hatchery-reared, juvenile red drum (*Sciaenops ocellatus*, Linnaeus): description of an ELISA, and predator-prey studies in nature. *J. Exp. Marine Biol. Ecol.*, in press.

2. **Hartmann, J.X., Poyer, J.C., Rossi, E.A., et al.** 1994. Summary of activities involving the development of antibody-based field identification kits for Atlantic Blue Marlin, White Marlin, and Atlantic Sailfish. *Intl. Commn. Cons. Atl. Tunas, Coll. Vol. Sci. Paps.* 45:5 16-526.
3. Hawkes, R., Niday, and E. Gordon, J. 1982. A dot immunobinding assay for monoclonal and other antibodies. *Anal. Biochem.* 119: 142- 147.
4. Huang, T., Marshall, M.R., Kao, K. *et al.* 1995. Development of monoclonal antibodies for red snapper (*Lutjanus campechanus*) identification using enzyme-linked immunosorbent assay. *J. Agric. Food Chem.* 43:2301-2307.
5. Ikegami, S., Mitsuno, T., **Kataoka, M., et al.** 1991. Immunological survey of planktonic embryos and larvae of the starfish *Asterina pectinifera*, obtained from the sea, using a monoclonal antibody directed against egg polypeptides. *Biol. Bull.* 181:95- 103.
6. **Manson, M.M. (Ed.)**. 1992. Methods in Molecular Biology: Immunochemical protocols. Vol. 10, **Humana** Press, Totowa, NJ.
7. Miller, K.M., Jones, P., and Roughgarden, J. 199 1. **Monoclonal** antibodies as **species-specific** probes in oceanographic research: examples with intertidal barnacle larvae. *Mol. Marine Biol. Biotech.* 1:35-47.
8. Richards, W.J., **Lindeman, K.C., L.-Shultz, J., et al.** 1994. Preliminary guide to the identification of the early life history stages of lutjanid fishes of the western central atlantic. NOAA Tech. Mem. **NMFS-SEFSC-345, 49p.**
9. **Robins, C.R.** and Ray, G.C. 1986. A field guide to Atlantic Coast Fishes of North America. Houghton Mifflin Co., Boston, 354 p.
10. **Sarver, S.K.,** Freshwater, D.W., and Walsh, P.J. 1995. Phylogenetic relationships of western atlantic snappers (Family Lutjanidae) based on mitochondrial DNA sequences. **Copeia**, in press.
11. Schultz, D.R. and Clarke, M.E. 1995. An immunological study of predation on hatchery-reared, juvenile red drum (*Sciaenops ocellatus*): preparation and assays of a “red drum-specific” protein for predator-prey experiments. *J. Exp. Marine Biol. Ecol.* 189:233-249.
12. Ward, B.B. 1990. Immunology in biological oceanography and marine ecology. *Oceanography* (April), 30-35.

HOW CONSUMER EDUCATION AFFECTS ATTITUDES TOWARD FOOD IRRADIATION

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A major obstacle facing the food irradiation industry is the lack of understanding by consumers as to the purpose for irradiation processing of perishable food items. Consumers have been exposed to much misinformation as to the **safety** and wholesomeness of irradiated food products. Research work over the past 50 years has proven low dose gamma irradiation (<10 kGY) to be effective in reducing pathogenic bacteria and extending shelf-life of such perishable food items as seafood, spices, fruits, vegetables, meat, and poultry. Extensive studies have shown that low dose gamma irradiation does not alter the wholesomeness of **fresh** products any more than thermal processing. Another misconception is that food producers will try to use irradiation to extend **shelf**-life of partially spoiled foods. This is exactly opposite of the irradiation processors purpose, in that they only submit the **fresh** products to irradiation. Another misconception is that irradiated products become radioactive. The effective **gamma** rays emitted from radiation sources in fact are much the same as X-rays. The rays pass through the food and dissipate into harmless lower energy particles. The purpose of this study was to determine the effect of food irradiation education on attitudes of a group of teens and young adults.

MATERIALS AND METHODS

Students, aged 16-24, were evaluated for their attitudes about **food** irradiation. Students aged 16- 17 were **from** a local public parish high school enrolled in second semester chemistry. Students aged 18-24 were University students, non food science majors enrolled in either basic Food Science or Food and Drug Law course.

Students were given an attitudinal **PreTest** in which they were asked general questions about the purpose and safety of food irradiation. After a period of 2-3 weeks, students were presented a general lecture and video, prepared by the Purdue Cooperative Extension Service, (1994). In this lecture, students were given general information about all food processes and where irradiation processing fit into the overall picture of food processing. The video also in picture form presented the general topic of food irradiation, showing how an irradiation plant is designed and what

irradiated foods look like. After the lecture and video, the students were given the same attitudinal test (**PostTest**).

Thirteen attitudinal questions were scored as Strongly Agree, Agree, Uncertain, Disagree, Strongly Disagree. One question on whether they would buy irradiated food products was scored as Yes or No. Analysis of mean variance were evaluated by SAS, 1987.

RESULTS AND DISCUSSION

Attitudinal questions follow with the pre and post test results. In all instances the mean attitudes about the benefit, safety, and wholesomeness of irradiated foods showed significant improvement ($p < 0.01$). Students were less concerned about the potential of irradiated foods causing cancer (74% to 27%); 84% said they would consider buying irradiated foods if available, up from 47% on pretest, and attitudes about the ability of irradiation to reduce the threat of foodborne illness were improved from 31% to 75%.

Evaluation of older consumers is currently under study to determine if consumer education, in general, will alter attitudes **and affect** buying behaviors.

REFERENCES

- Josephson, E.S. and Peterson, M.S. 1982. *Preservation of Food by Ionizing Radiation*, Vol. I, II, III, Boca Raton, FL: CRC Press, Inc.
- Purdue Research Foundation. 1994. *The future of food preservation: Irradiation*. Department of Foods and Nutrition, Agricultural Communication Services, Purdue University, West Lafayette, IN. 47906
- Urbain, W. M. 1986. *Food Irradiation*. academic Press, Inc., Orlando, pp. 1-35 1.

BIGHEAD CARP: ASSESSMENT OF TWO POTENTIAL CANNED PRODUCTS

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INTRODUCTION

The catfish, bait minnow and Chinese carp industries in the U.S. were all born in Arkansas. Arkansas has over 65,000 acres of water devoted to aquaculture production (McNulty, 1996). Arkansas today trails only Mississippi in acreage and sales of catfish and leads the nation in bait minnow production and Chinese-carp-hatchery production (USDA, 1995). Commercial fish farming has a significant impact on the economy of the state and surrounding Delta region and has a potential for growth that far exceeds its present economic importance.

Alternative fish products and alternative species would allow Delta fish farmers to diversify production in their ponds as a risk management strategy. However, little information is available on the marketing potential of alternative species such as **bighead carp** (*Hypophthalmichthys nobilis*). Carp, as a group, make up the greatest production of fish by weight from worldwide aquaculture (Billard and Perchee, 1993) with **bighead carp** ranking third in total aquaculture output (Engle, 1992). **Bighead carp** have been raised in Arkansas since the 1970's as a polyculture species in efforts to improve water quality in catfish ponds. Although other countries have established markets for this freshwater fish, **bighead carp** have not been marketed on a wide scale in the U.S. Arkansas fish farmers have sold **bighead** for resale in Asian ethnic markets as a live product. This market is easily saturated resulting in wide fluctuations in supply and price. Therefore, a higher-volume market outlet, such as a cannery, would provide stability and a constant market for **bighead**. Consumer acceptance of potential, **canned bighead** carp product forms is relatively unknown.

Regardless that the U.S. has not been a very sophisticated marketplace for canned fish products, with the exception of specific ethnic consumer demands, there may be room in the marketplace for new canned products made from non-traditional fish species (Regenstein and Regenstein, 1991). Despite the emergence of frozen, convenience products and the more ready availability of **fresh** seafood, canned seafood is still consumed by more households than any other fish product form (Thomas and Engle, 1995). The most popular canned fish in the U.S. are salmon, **tuna**, and sardines in oil or water packs (Regenstein and Regenstein, 1991). In 1987, the U.S. imported 10.8 million cases of (105,000 tons) of **canned** tuna (Parks et al., 1990).

silver carp (*Hypophthalmichthys molitrix*), closely related to bighead carp, was evaluated in the fresh and canned form. In market studies conducted at Auburn University, silver carp marketed as a fresh product was not well accepted (Crawford et al., 1978; Engle, 1978). Engle (1978) reported that only 14% of people who purchased silver carp would repurchase it. The foremost reason for consumer dislike of this fish was the abundance of small intramuscular bones. However, silver carp were more accepted by consumers when canned (Woodruff, 1978).

Early market studies indicated that fresh bighead carp was readily accepted for its taste, but, as with silver carp, the fresh bighead was deemed too bony (Engle, 1992). Since canning softens bones, a canned bighead product was evaluated in a limited 1992 study by researchers at UAPB (Thomas and Engle, 1995). They assessed the attitudes of 471 Arkansas consumers and concluded the canned product had market potential. Respondents to the informal product evaluations suggested that the low-fat content of the product and its taste appeal were positive attributes (Engle and Kouka, 1995).

The primary goal of this on-going study is the development and evaluation of a value-added, canned fish product from the alternative freshwater fish, bighead carp. The specific objective of this phase of the study was to determine sensory quality, acceptability and market potential of two products prepared from either steam or oven cooked carp prior to canning.

MATERIAL AND METHODS

Carp

Six hundred, seventeen kg of bighead carp ranging in size from 3.18 to 4.99 kg each were harvested in November, 1994. The carp had been grown for approximately a year in polyculture with channel catfish in experimental ponds at the University of Arkansas at Pine Bluff, Pine Bluff, AR. The carp were immediately transported alive to a nearby fish-dressing area where they were stunned, beheaded, eviscerated, skinned and fins and tails removed to yield primal loin cuts. The loins were washed to remove extraneous materials and packed in ice for transport to the University of Arkansas Food Science Department in Fayetteville, AR, for processing into hybrid-type canned products having properties that fall between those of a totally boneless product, like tuna, and those of a fully boned product, like salmon.

Precooking Treatments and Flesh Removal

For Purposes of this study, the term "precook" means heated/cooked by some means prior to flesh removal and can filling for subsequent thermal processing (retorting). Approximately 106 kg of loins (approximately 2.2 kg ea) were removed from the ice and divided into two equal lots representing the following precooking treatments: steam-cooked and oven-cooked. For both precooking treatments, loins were placed belly-side down on perforated stainless steel trays and heated either in an atmospheric steam cabinet for 30 min or convection oven at 177°C for 60 min. The duration of heating for the two precooking methods was determined as the amount time required for approximately 85% of the loins to reach a backbone temperature of 77°C. After precooking, loins were placed in a walk-in cooler at 5°C for 7 hr to firm the muscle tissue. After

firming, the flesh was separated into halves from both sides of the backbone. The portions were again divided horizontally and the red meat removed completely. The loins were “polished” by scrapping off the remaining flesh from the ribs and the backbone. Edible flesh was removed from ribs and backbones in chunks as large as possible. Weights of the various meat and waste portions from each treatment were determined to enable yield calculations.

Thermal Processing

Three hundred, thirty grams of meat was packed into 300 X 407 C-enamel cans. The cans were filled with a hot (93°C) 2% NaCl brine, exhausted for 5 min in a steam tunnel and then immediately seamed with a commercial can closer. Two retort cooks were carried out - one for each of the two precook treatments. Approximately 40 cans from each treatment were thermally processed in a stationary retort. Heat penetration tests were conducted in duplicate for each product according to the procedure described by Kattan et al. (1989). All cans were processed at 121.8°C for 85 min (F_0 values 30 to 45).

Proximate Composition

The contents of the canned samples were drained through a 0.60 mm stainless steel sieve for 10 min. Raw loin chunks (approximately 20 g ea) from several loins and drained samples were homogenized in a blender and then analyzed for moisture, protein (%N X 6.25), crude lipid, and ash following standard AOAC methods for meat analyses.

Consumer Perception Testing

Sample preparation. The contents of the canned samples were drained for 2 mm on a U.S. No. 8 sieve. Samples were separated gently with a fork to provide pieces of similar size for presentation to panelists. Portions of approximately 60 g each were placed into cups for presentation to the panelists.

Consumer trials. The two products were evaluated by 90 consumers, all of whom had indicated that they like salmon and tuna-style canned fish products. Sensory and acceptance testing was conducted by the Sensory Analysis Group, Food Science Department, University of Arkansas. Panelists were recruited through parent-teacher organizations in the northwest portion of Arkansas. Prior to evaluation, the ballot and the evaluation procedures were discussed with each panelist to familiarize them with the evaluation process. To avoid any bias toward carp, panelists were not told they would be evaluating bighead carp. Panelists were only told the product was a freshwater fish with commercial potential. Each panelist was then seated in a sensory booth or an individually partitioned area to evaluate the products. The products were presented together in balanced random order and evaluated under GE Chroma 50 lamps that provide natural daylight illumination. The samples were served at room temperature. After the evaluations were completed, panelists were asked to return to the lobby area of the facility until their ballots could be checked to ensure all questions had been answered.

Sensory Analysis. Sensory data were analyzed as described in Mailgaard, et al. (1991). Analyses of Variance were performed for attribute liking rated on 7-point hedonic scales for **appearance**, overall liking, and flavor.

Acceptability and Marketing Attitudes. Panelists were asked to compare the carp products with similar canned fish products and rank them as better than, equal to, or not as good as the other products. Purchase intentions were estimated by asking open-ended questions related to willingness to pay as much as for the carp as other similar products.

Attribute Diagnostics. To determine product attribute direction for change, frequency distributions were calculated for 5-point Just Right scales for color, aroma, salt, firmness and moistness (Mailgaard, et al., 1991). Categories of not enough and too much were compared to determine direction for change.

RESULTS AND DISCUSSION

Proximate Analysis

The proximate composition of raw carp loins and the canned products are presented in Table 1. Heating causes various physical and chemical changes in canned fish products (Lazos, 1995). There was a decrease in moisture after canning which probably could be attributed to the moisture loss experienced during the precooking step as well as to protein denaturation during thermal processing (Table 1). The precooking treatment affected the water content of the canned products with moisture loss being more profound in the oven-cooked samples compared to the steam-cooked samples. During precooking, loins lost approximately 20% weight in the dry conditions of the oven compared to losing approximately 13% in the wet environment of the steam cabinet (data not shown). Obviously, the meat rehydrated, to some extent, in the can since moisture contents of the two canned products didn't vary more than 4.5% (Table 1). Percent lipid, protein and ash of the canned carp varied little from the raw carp. Protein and lipid contents were slightly greater in the two canned products which most likely can be attributed to moisture loss during the precooking step prior to canning.

Table 1. Proximate composition of raw and canned carp.

Carp Form	Percent			
	H ₂ O	Protein	Lipid	Ash
Raw	83.0	16.5	0.7	1.7
Oven-Baked	78.6	16.9	ct.8	1.7
Steam-Baked	80.9	16.6	0.7	1.7

Liking/Acceptability

Hedonic sensory scores, with the percentage of panelists who rated the canned carp as like very much or like moderately, for appearance, flavor and overall liking are presented in Table 2. Method of precook significantly affected the hedonic scoring of the two products, but overall acceptability was extremely good for both canned carp products. The worst-case, average hedonic score for either product was only 3.08 (7=dislike very much) for the oven-cooked product category of appearance. Panelists consistently preferred steam-cooked carp over the oven-cooked carp. Sixty-four percent of panelists judged the appearance of the steam-cooked carp superior to the oven-baked carp (Table 2). Likewise, over 50% of panelists consistently ranked the

Table 2. Liking/Acceptability - Mean hedonic scores^c and percentages () of panelists who rated canned bighead carp as like very much or like moderately.

Precook	Appearance	Flavor	Overall Liking
Oven	3.08 ^a (43%)	3.02 ^a (47%)	3.07 ^a (48%)
Steam	2.47 ^b (64%)	2.47 ^b (58%)	2.52 ^b (54%)

^{a-b}Means within columns followed by the same superscript are not different ($P>0.05$) by LSD test.

^cHedonic scale, 1 =like very much; 7=dislike very much.

steam-cooked carp as like very much or like moderately for flavor and overall liking compared to the oven-cooked products. Even though steam-cooked carp was more preferred, Table 2 reveals that the oven-cooked carp also was well liked since it ranked only 6 percentage points below steam-cooked carp for the attribute overall liking.

Comparisons to Other Canned Fish Products

Panelists were asked to compare the two carp products with canned mackerel, salmon and tuna. As shown in Table 3, panelists indicated that the bighead carp compared most favorably with tuna and least favorably with mackerel. Over 60% of panelists indicated that either carp product was better than or equal to tuna compared to 45 and 38 % for salmon when compared to steam and oven-cooked carp, respectively. The low comparability for mackerel may not be meaningful since 77% of the panelists indicated that they were not familiar (don't know) with canned mackerel. Steam-cooked carp garnered higher "better than or equal to" scores compared to oven-cooked carp when compared to either salmon or tuna. Exit interviews with panelists provided insight into why many felt that the carp did not compare favorably with salmon. The carp products, it seems, were deemed too bland or too mild for valid comparison with salmon. Panelists expected a salmon-type product to have strong, fishy odors and flavors.

Table 3. Percentage of panelists who indicated that **canned** bighead carp was better **than** or equal to other products - Comparison of precooking treatments.

Comparison Product and Precook	Better Than or Equal to	Not as Good	Don't Know
Mackerel			
Oven	20	3	77
Steam	18	4	77
Salmon			
Oven	38	39	24
steam	45	32	24
Tuna			
Oven	62	38	0
steam	67	33	0

Purchase Intentions

Attitudes about products obviously dictate purchase intentions. For similar-sized cans, **panelists** were asked whether they would be willing to pay as much for the bighead carp as other **canned fish products**. Responses to such bottom-line type questions provide insight into overall **acceptance** and attitude. Willingness to pay as much for a new product as traditional canned products is viewed as an overall positive for the product concept. Table 4 presents the **percentages** of panelists willing to pay as much for the two canned carp products as canned mackerel, salmon and tuna. Fifty-two percent indicated they would not pay as much for the oven-cooked carp as mackerel while 55% would pay as much as mackerel for the steam-cooked carp (Table 4). It should be noted that only 50 panelists answered the mackerel section of the willingness to buy questionnaire. With that and the fact that 70% of the panelists indicated they were unfamiliar with mackerel (Table 3), any comparisons with mackerel may not be valid. Obviously, some panelists unfamiliar with mackerel chose to guess rather than base their answer on experience. Comparisons with salmon and tuna should be more meaningful since 76% and 100% of **panelists** were familiar with salmon and tuna, respectively (Table 3). As Table 4 presents, 58 and 54% were not willing to pay as much for oven or steam-cooked carp as salmon, respectively. The **strongest response** on willingness to pay was the comparison with tuna. Sixty-three and 67% said they would pay as much for oven or steam-cooked carp as **tuna**, respectively. These **results are encouraging since** tuna is the highest-volume canned fish product sold in the **U.S.** Table 4 **data indicate** there may be market potential for either of the **canned** carp products if **either could** be priced **competitively** with canned tuna.

Table 4. Percentage of panelists willing (unwilling) to pay as much for canned bighead carp as for other canned fish products- Comparison of carp precooking treatments.

Comparison Product and Precook	Yes	No
Mackerel		
Oven	48	52
Steam	55	45
Salmon		
Oven	42	58
Steam	46	54
Tuna		
Oven	63	37
Steam	67	33

Directional Attribute Diagnostics

Because canned bighead carp likely would be in direct competition with canned tuna, a well liked product, it's important that the potential "gold standard" carp product has the quality and sensory attributes most desired by consumers. Improvements to the carp products must be based on the reasons panelists may have preferred or rejected certain sensory attributes. Therefore, attitudes about product attributes were determined using just right scales as described by Meilgaard et al. (199 1). Just right scales allow assessment of the intensity of a particular attribute relative to some mental criterion of the panelist's ideal for that attribute.

Panel responses to the just right scales as applied to the attributes, color, aroma, salt level and firmness are presented in Table 5. Responses were mixed between the two carp products with respect to suggested directions for change for the attribute color.

Table 5. Percent response of panelists in each category for color, aroma, salt level and firmness - Comparison of carp precooking treatments.

Attribute and Precook	Much too Little	Somewhat too Little	Just Right	Somewhat too Much	Much too Much
Color					
Oven	2	11	54	31	1
Steam	10	21	67	1	1
Aroma					
Oven	1	11	51	30	7
Steam	3	16	69	10	2
Saltiness					
Oven	13	29	44	12	1
Steam	22	32	42	3	0
Flavor					
Oven	4	24	53	14	3
Steam	14	28	56	2	0

^ajust right scale used, too light to too dark

^bjust right scale used, too weak to too strong

^cjust right scale used, not salty to too salty

^djust right scale used, too soft to too firm

Fifty-four percent of the panelists indicated the oven-cooked carp was just right in color intensity while 67% felt the steam-cooked carp was just right in color. Interestingly, panelists felt the oven-cooked carp was too dark (3 1% somewhat too dark and 1% much too dark) and the steam-cooked too light (2 1% somewhat too light and 10% much too light). Table 5 presents that panelists believed the oven-cooked carp smelled somewhat too strong (30%) while a majority of panelists deemed the steam-cooked carp just right (69%) to somewhat too weak (16%) in odor. Directional responses further indicate that panelists would have preferred saltier and firmer products regardless of cooking method (Table 5).

CONCLUSIONS

Canned bighead carp appears to have potential as a canned, freshwater fish product. Overall consumer acceptance of the product concept was positive. Canned bighead carp has several desirable characteristics which include a low fat content (<1%), a very mild taste and a white

appearance similar to albacore (white) tuna. Acceptance varied with method of precook. Sensory scores indicated that the steam-cooked carp were consistently preferred over the oven-cooked carp. Sixty-four percent of panelists judged the appearance of the steam-cooked carp superior to the oven-baked carp. Likewise, over 50% of panelists consistently ranked the steam-cooked carp as like very much or like moderately for flavor and overall liking compared to the oven-cooked products. Over 60% indicated that either canned carp product (steam-cooked or oven-cooked) was better than or equal to canned tuna. Likewise, over 60% of panelists indicated a willingness to pay as much for either carp product as canned tuna. Directional responses using just right scales demonstrated that steam-cooking produced the most desirable color and aroma, but panelists indicated both products should have more salt and be firmer. Further product development efforts will focus on increasing product firmness, evaluating a “smoked” line extension, determining processing costs and conducting break-even analyses.

REFERENCES

- Billard, R. and G. Perchec. 1993. Systems and technologies of production and processing for carp. In *Workshop on Aquaculture of Freshwater Species (except salmonids)*, P. Kestemont and R. Billard (Ed.), 1-5, European Aquaculture Society Special Publication No. 20, Ghent, Belgium.
- Crawford, K.W., D. R. Dunseth, C. R. Engle, M. L. Hopkins, E. W. McCoy and R. O. Smitterman. 1978. In *Culture of Exotic Fishes Symposium Proceedings*, R.O. Smitterman, W.L. Shelton, J.H. Grover (Ed.), 240-257, Fish Culture Section, American Fisheries Society, Auburn, AL.
- Engle, C. R. 1978. Preliminary market tests of several exotic fish species. MS. Thesis, Auburn University, Auburn, AL.
- Engle, C. R. and P. J. Kouka. 1995. Potential consumer acceptance of canned bighead carp: a structural model analysis. *Marine Resource Economics* 10: 101 - 116.
- Engle, C. R. 1992. Market tests and consumer acceptance of fresh bighead carp. *Arkansas Aquafarming* 10 (1):6.
- Kattan, M. W., T. J. Siebenmorgen, D. R. Davis and W. G. McGlynn. 1989. A computerized system for evaluating the effectiveness of a thermal canning process. *Arkansas Farm Research* 38(5):5.
- Lazos, E. S. 1995. Thermal processing of freshwater cyprinids: chub (*Leuciscus cephalus*) and ide (*Leuciscus idus*). *J. Aquatic Food Product Technology* 4(1): 31-50.
- Meilgaard, M., G. V. Civille and B. T. Carr. 1991. Affective tests: attribute **diagnostics**. In **Sensory Evaluation Techniques, 2nd Edition**, 201-237, CRC Press, Boca Raton, FL.

McNulty, T, 1996. Personal communication. State of Arkansas, Aquaculture Coordinator, Little Rock, AR.

Parks, W. W., P. J. Donley and S. F. Herrick. 1990. U. S. trade in canning. *Mar. Fish. Rev.* 52: 14-22.

Regenstein, J. M. and C. E. Regenstein. 1991. Other forms of fish preservation and packaging. In *Introduction to Fish Technology*, J.M. Regenstein (Ed.), 120-138, Van Nostrand Reinhold, New York, NY.

Thomas, M. and C. R. Engle. 1995. Consumer acceptance of canned bighead carp: a new freshwater fish product. Arkansas Agricultural Experiment Station Report Series 328.

USDA. 1995. Aquaculture - situation and outlook report. Economic Research Service, AQUA-13, Washington, DC.

Woodruff, V. C. 1978. Marketability of canned silver carp. MS. Thesis, Auburn University, Auburn, AL.

PROPERTIES OF SURIMI MADE FROM TILAPIA

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INTRODUCTION

Surimi is a Japanese Term for mechanically deboned fish meat washed with water and mixed with cryoprotectants. Washing step removes water-soluble proteins, fats and undesirable materials such as blood, pigments, and odorous substances, enzymes, and trimethylamine oxide (TMAO). It increases the concentration of myofibrilla proteins (actomyosin), which improve gel-forming ability and elasticity (Lee, 1984; Okada, 1985).

Surimi is an intermediate product for further production of fabricated seafood products such as imitation crab legs, scallops, and shrimp. It can be substituted for a variety of traditional animal and vegetable proteins (Lee, 1984; Wu, 1992). The overall amino acid score of surimi is similar to those of beef and turkey (AFDF, 1987).

At present time, 90% surimi is made from Alaska pollack with small amounts made from croakers and sharks (Piggot, 1986). Little efforts have been made to produce surimi from freshwater species. Tilapia is the most important freshwater aquaculture species worldwide. In Egypt, this fish is abundant and low market value. Consumption of fish in Egypt is not very high. Processed fish products are very limited. To use these species, the objectives of this study were (1) to determine the yield and chemical quality and (2) to exam the functional properties of **surimi** made from tilapia.

MATERIALS AND METHODS

Preparation of tilapia surimi

Forty-five kg of life tilapia (*Oreochromis niloticus*), averaging 30.85 cm in length and 4564.00g in weight, were obtained from Dekalb Farmers Market, Atlanta, Georgia. Fish were removed from a water tank and filleted. Both fillets and **frames** were packed on ice and transported to Department of Food Science and Technology, University of Georgia, Athens, GA. Surimi was prepared from fillets and frames using the method of Park et al.(1990) with some modification (Figs. 1 and 3). Minced meat was recovered **from** fillets and frames using a deboner (Yanagiya Machinery Works, Ltd. Japan) with a 4-mm hole drum. The minced fish were immediately washed four times

in a stainless steel tank (50L) with iced water at a ratio of 1 part flesh to 3 parts water (w/w). Water was used for the fourth wash cycle contained 0.15% (w/w) NaCl to facilitate de-watering. Hand whipper was used to stir the slurry for 5 min and excess water was removed between washes using cheesecloth. A basket centrifuge (Wastem States, Hamilton, OH) was used for the final de-watering to the extent that water was no longer visibly being extracted from the washed mince. Raw surimi was either directly vacuum-packaged or chopped with cryoprotectants (4% sucrose, 4% sorbitol and 0.25% sodium tripolyphosphate, STPP) for 2 min using a Hobart silent cutter and then vacuum-packaged. Oxygen barrier-bags (Surlyn, oxygen transmission rate = $5\text{cc/m}^2/24\text{hr/atm}$, at $4.4\text{ }^\circ\text{C}$ and 0% Rh, Cryovac Co. Duncan, SC) and a Multivac AG 900 vacuum packaging machine (Multivac, Sepp Hagggenmuller KG, Germany) were used to pack all samples.

Proximate Composition

Moisture, crude protein, fat and ash content were determined according to AOAC (1984) methods.

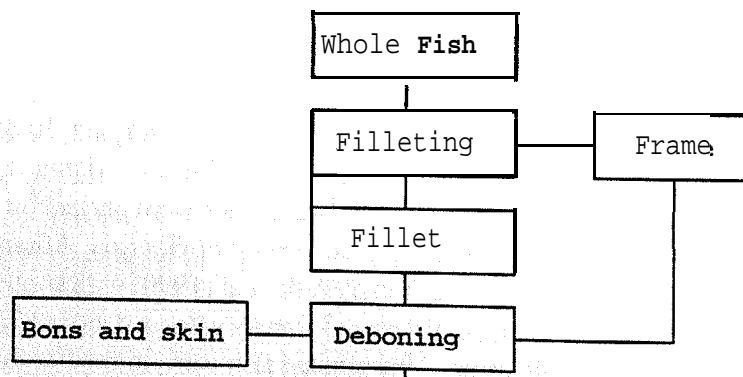
Protein in Washing Water

Washing water in different washing cycles were filtrated using Whatman No.2 filter papers. The filtrate was used for protein analysis (Douglas-schwarz and Lee, 1988).

Expressible Water

A 3mm thick and 20mm diameter slice of surimi gel was pressed between four sheets of Whatman No. 1 filter paper under a weight of 10.8kg for 1 min. The weight loss of a gel slice after pressing was expressed as percent of the original sample weight (Hennigar et al., 1988).

Expressible water for a surimi sample was determined according to Alvarez et al. (1992). A 2g of a surimi sample was folded with one sheet of filter paper Watman No. 1 and centrifuged at 3000 rpm for 15 min at room temperature (Servall angel centrifuge type SPX., Ivan Servall Inc. Norwalk, Corn.). Prewieghed nylon screen was placed between the surimi sample and the filter paper sheet. This was used to aid in removing the sticky "cake" rapidly from the filter paper after centrifugation (Weinberg et al., 1984). Values reported represent an average of six samples per treatment.



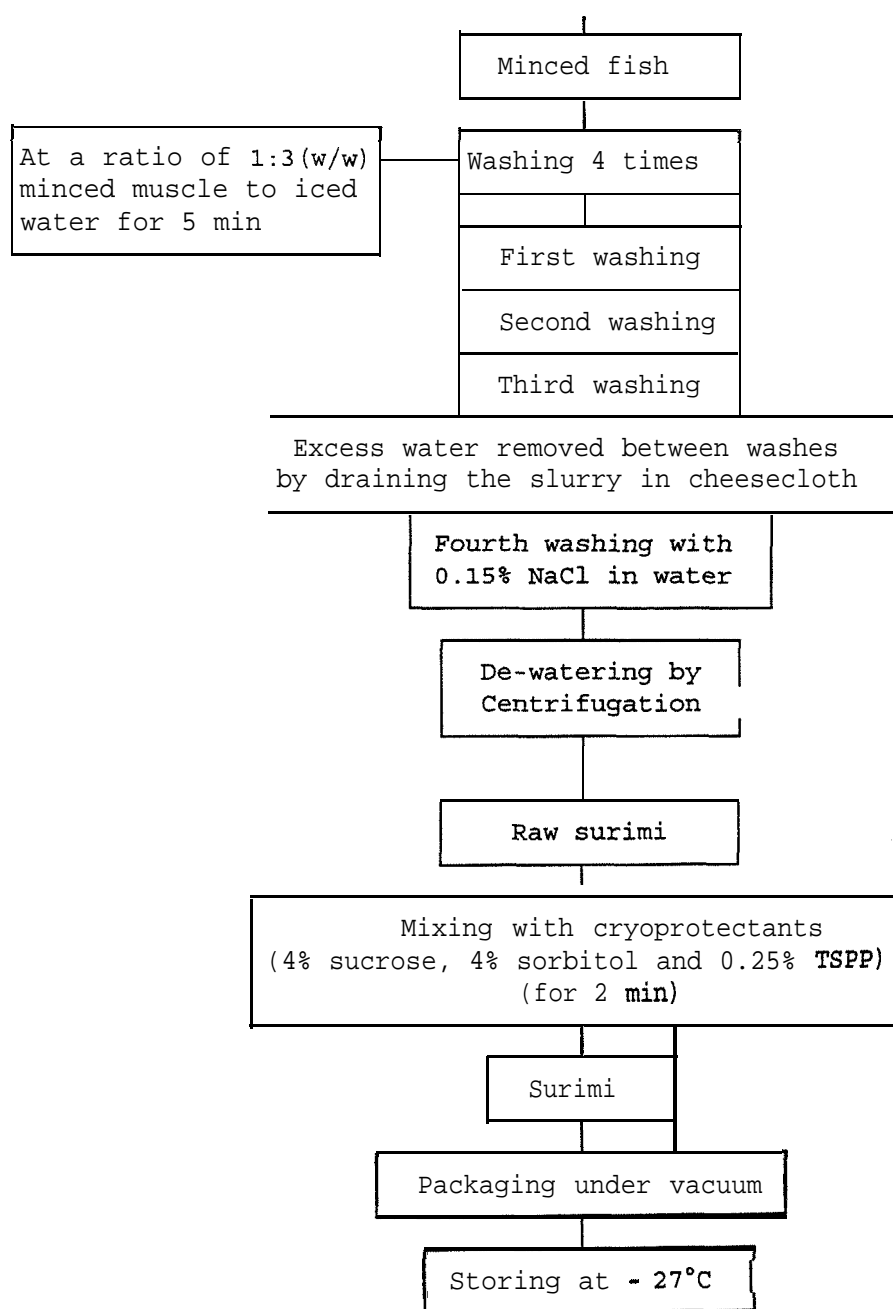


Fig. 1 A flowchart of processing surimi from tilapia

Hunter Color Values

Hunter color values (L, "a", "b") of fish mince during washing steps and final surimi products were measured using a colorimeter (Minolta Chroma Meter CR-200, Minolta Camera, Ltd., Osaka, Japan). The value L was the lightness ranged from 0 to 100; "a" was chromaticity where positive value indicating redness and negative value indicating greenness; while positive value of "b" indicating yellowness and negative value indicating blueness. Three random spots on each sample were measured and the average data were recorded.

Mineral Content

Five macro-elements (Na, Ca, Mg, P, and K) and fourteen micro-elements (Zn, Cu, Fe, Si, Co, Mn, Mo, Ni, Pb, Sr, Ba, B, Al, and Cr) in fish mince and surimi were determined. One g of homogenized sample was weighed into a 100-ml beaker and 5 ml concentrated nitric acid was added. After the sample was dried on a hot plate, the beakers were placed in a muffle furnace at 500 °C for 4 hrs. The ash was then taken up in 10 ml 20% aqua regia. The resulting solution was assayed by inductively coupled plasma (ICP) emission spectrometry using a Thermo Jarrel-Ash 955 AtomCounter ICP Spectrometer.

Amino Acid Content

A 0.2g sample (contained 30 mg crude protein) was hydrolyzed by adding 180 ml 6N HCl under nitrogen for 24hr in a 500ml flat bottom flask connected with a condenser. The hydrolysate was filtered through Whatman No. 1 filter paper and distilled water was added to adjust the volume to 200 ml.

20 ml of hydrolysate was placed into a 500 ml round bottom flask evaporated under a vacuum at 60 to 65 °C, 15 ml of diluting buffer (Sodium citrate; pH 2.20) was added to dried content and sufficient volume was injected in the Beckman high performance amino acid analyzer system 6300, (Beckman Instruments Inc., Palo Alto, CA)

Measurement of Gel Strength

The gel was prepared by comminuting surimi with 2% salt in a food processor (Regal Lamachinei, VB., VA) at 0°C for 10 min (Lee and Chung, 1989). The Moisture content of surimi was adjusted to 78% by adding ice during chopping. The resulting paste was immediately vacuum packaged in oxygen barrier-bags. The paste was stuffed into both stainless steel tubes (1.87cm id and 16.5cm length) and plastic casing (3cm diam) with minimum incorporation of air bubbles and sealed at both ends. The interior of the tubes was coated with a thin film of PAM (American Home Food Inc., Madison, NJ). The tubes and plastic casings were submerged vertically in 40°C water bath for 20 min and then in 90°C water bath for 15 min. The tubes and plastic casings were kept at 40°C for 12 min and 17 min, respectively before calculating the setting time. The tubes and plastic casings were kept at 90°C for 10 min and 13 min, respectively before calculating the cooking time. The temperature in the center of the gels was monitored in the tubes and casings using small thermocouple probes. Immediately after heated the tubes and casings were cooled in a mixture of ice and water for 10 min. Gels were removed from tubes and casings and kept in sealed plastic bags at 4°C for overnight until testing (Fig. 4).

Measurement of Strain and Stress at Failure

Surimi gels (1.87cm dia.) were removed from the refrigerator and warmed to the room temperature. Plastic disks were glued onto the ends of each sample in 2.87cm length with cyanoacrylate glue. Samples were cut into dumbbell-shaped specimens with a final minimum diameter 1cm using a machine (Accu-tool corp, cary, NC). The dumbbell-shaped samples were placed in a modified Brookfield viscometer (Model DV-I) with

a torsion fixture attached and twisted at 2.5 rpm until failure (Fig. 5). Stress and strain at failure were calculated from the data using the equation given by Hamann (1983).

Penetration Test ("Punch" Test)

Surimi gels (3cm dia.) were brought up to room temperature and cut into 4cm length. The sample was placed on a texture analyzer (Voland Texture Analyzer TA- 1000, Voland Corporation, Hawthorne, NY) and a 5mm ball probe was driven into the flat top of the surimi sample. The speed of the probe was 1.0mm/sec, the depth was 23mm and the chart speed was 10.0cm/min. Breaking force (g) and breaking distance (cm) were recorded by using the flat-bed record (Linseis L6512, Linseis Inc., Princeton-Jet., NJ) (Fig. 6).

Gel strength (g \times cm) = force (g) \times breaking distance (cm).

Folding Test

A surimi gel slice (3cm diameter and 3mm thick) was folded by thumb and forefinger. Five stages were used to evaluate the sample (Kudo et al., 1973):

AA=5 (no cracks on folding twice), A=4 (no cracks showing after folding in half), B=3 (crack gradually when folding in half), C=2 (crack immediately when folding in half), and D=1 (breaking by finger pressure).

Data Analysis

Data were analyzed with the GLM (General Linear Model) program using statistical analysis system (SAS, 1987). Mean values were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Surimi Yield

Table 1 showed that the yields of surimi from tilapia (18.99%) and grass carp (18.26%) were lower than that of Alaska pollack (24.00%). Hustings (1989) reported that lower surimi yield may be due to the loss of water soluble protein during wash steps and of some insoluble protein during the draining of slurry. Park et al. (1990) found that a higher yield of protein recovery (51.59%) and ease dewatering due to the pre-rigor tilapia.

The yield of minced flesh from tilapia frames without head was 42.50% and that from the whole fish was 5.63% (Table 2). The yield of surimi from the frame without head was 10.82% and that from whole fish was 1.43% that equal 6.965 of the surimi processed from fillets.

Protein Loss During Washing

Most of the water-soluble proteins were removed in the first washing cycle (Table 3). This data agrees with the report of Yean (1993) indicating that most soluble protein loss occurred in the first wash (2.7g protein/L¹) during processing surimi from fresh Threadfin bream (*Nemipterus tolu*). Eid et al. (1991) also reported that the leached out protein percentage in six fish species (catfish,

Table 1 - Yield of surimi made from tilapia and grass carp

	Tilapia		Grass Carp	Alaska pollack ^a
	flesh	frame	%	
Whole fish	100	-	100	100
Fillets to whole fish	39.74	-	47.19	47.00
Minced flesh to whole fish	30.90	5.63	35.21	-
Minced flesh to fillet	77.78	-	74.61	-
Washed mince to whole fish	18.99	1.32	18.26	22.00
Washed mince to fillet	47.78	-	38.71	-
Washed mince to minced flesh	61.43	-	51.88	-
Surimi^b to whole fish	20.54	1.43	19.76	24.00
Surimi to fillet	51.69	-	41.88	-
Surimi to minced flesh	66.46	-	56.13	-

a from AFDF, (1987)

b **Washed** minced flesh mixed with cryoprotectants (4.00% sorbitol, 4.00% sucrose and 0.25% sodium triplyphosphate (STPP))

Table 2 - Yield of surimi made from tilapia frames

	Tilapia Frame %
Frames with head to whole fish	36.20
Frame without head to whole fish	13.25
Minced flesh to whole fish	5.63
Minced flesh to frame with head	15.55
Minced flesh to frame without head	42.50
Washed mince to whole fish	1.32
Washed mince to frame with head	3.66
Washed mince to frame without head	10.00
Washed mince to minced flesh	23.53
Surimi* to whole fish	1.43
Surimi to frame with head	3.95
Surimi to frame without head	10.82
Surimi to minced flesh	25.46

* Washed minced flesh mixed with cxyoprotectants (4.00% sorbitol, 4.00% sucrose and 0.25% sodium tripolyphosphate (STPP))

Table 3 - Protein content in washing water during surimi processing from minced **fillet** and **frame** of tilapia and fillet of grass carp

Samples	Tilapia meat ^b	Tilapia frame ^a	Carp meat ^b
Number of washing	Protein g/100ml		
First wash	0.81	0.95	0.54
second wash	0.29	0.26	0.21
Third wash	0.11	0.07	0.10
Fourth wash	0.05	0.06	0.05

n=2

a = Water:minced flesh (3: 1 W/W)

b = Water:minced flesh (8:1 V/V)

Table 4 - Proximate composition of tilapia and grass carp surimi

Samples	Moisture %	Protein %	Fat %	Ash %	Carbohydrate %	Expressible water %
Minced fillet*	77.87d ±0.18	16.23ab ±0.94	3.87c ±0.34	1.20a ±0.03	0.00	31.95b ±0.66
Minced frame*	79.51c ±0.23	14.32cd ±0.39	5.15b ±0.08	0.99b ±0.03	0.00	42.69a ±0.87
Washed meat	81.08b ±0.52	16.27ab ±0.79	2.56d ±0.06	0.58c ±0.04	0.00	26.50c ±0.20
Surimi fillet*	73.91f ±0.89	14.81cd ±0.54	2.31d ±0.27	0.55c ±0.02	8.00	20.64d ±1.20
Surimi frame*	72.94g ±0.76	15.36cb ±0.92	2.46d ±0.19	0.53c ±0.02	8.00	14.54e ±1.46
Carp meat	74.68f ±0.18	17.33a ±1.13	6.60a ±0.25	1.07b ±0.03	0.00	31.37b ±1.87
Washed mince	82.64a ±0.19	14.84cd ±0.59	1.18e ±0.12	0.23d ±0.02	0.00	27.07c ±2.27
Carp surimi	76.63e ±0.15	13.45d ±0.07	0.78f ±0.04	0.56c ±0.19	8.00	20.63d k2.44
Alaska pollack surimi ^a	76.00	16.00	0.20	0.34	7.50	NA

a from AFDF, (1987).

n=3

* Samples prepared from tilapia.

abcdefg Means in a column with different letters are different (p< 0.05).

Table 5 - Change of Tilapia and Grass Carp meat color during surimi processing

Samples	"L"			"a"			"b"		
	minced fillet*	minced frame*	minced carp	minced fillet*	minced frame*	minced carp	minced fillet*	minced frame*	minced carp
Minced flesh	52.05a ±0.29	49.11b ±0.65	52.62a ±1.44	+2.3 lc ±0.52	+7.33b ±0.91	+9.36a ±0.69	+2.89b ±0.58	+5.52a ±0.38	+6.03a ±1.07
First wash	59.09b ±1.57	54.50c ±0.39	62.29a ±1.08	+0.69c ±0.19	+1.45b ±0.13	2.31a ±0.18	+1.53c ±0.38	+2.81b ±0.41+	+4.05a ±0.71
Second wash	61.13b ±1.34	55.87c ±1.18	66.18a ±0.65	-0.70b ±0.25	-0.29a ±0.09	-0.88b ±0.13	-1.21b ±0.63	-0.28a ±0.18	+0.33a ±0.12
Third wash	61.73b ±1.09	58.24c ±1.47	66.20a ±0.77	-0.99a ±0.20	-0.87a ±0.41	-1.23a ±0.19	-1.40a ±0.93	-1.62a ±0.32	-0.53a ±0.42
Fourth wash	64.12b ±0.68	65.78b ±1.48	70.27a ±1.77	-0.62a ±0.17	-0.82a ±0.24	-1.07a ±0.46	+1.44a ±0.86	-1.86b ±0.57	+1.56a ±0.60
Surimi	70.68a ±1.27	70.46a ±1.99	71.40a ±0.83	-0.83a ±0.11	-0.68a ±0.15	-1.24b ±0.12	+2.68a ±0.54	+3.07a ±0.94	+2.41a ±0.48

n = 3

* Samples prepared from tilapia

abc Means in a row for each group of L, a, and b with different letters are different (p < 0.05).

goatfish lizardfish, ponyfish, therapon, and trevally) catch from Arabian Gulf was ranged from 30% to 58% during surimi processing.

Proximate Composition

The composition of flesh though processing surimi was changed by removed of water-soluble protein components, lipids and minerals (Table 4). Surimi from Tilapia fillet and frame were lower ($p < 0.05$) moisture content and higher fat content than that of Carp and Alaska pollack surimi. Tilapia and Carp surimi have lower protein content than Alaska pollack, but tilapia surimi has higher protein content than that of Carp surimi. Park et al. (1990) stated that the moisture and protein contents of surimi processed from tilapia in pre-rigor, in-rigor and post-rigor stages were 70.53% and 20.27%, 75.63% and 15.42%, and 75.03% and 16.57%, respectively. The lipid content of minced flesh from tilapia fillet and frame and grass carp was reduced by 40.31%, 52.23% and 88.18%, respectively during processing surimi. Ash content also reduced by 54.17%, 46.46% and 47.66%, respectively. Chang-Lee et al. (1990) reported that lipid and ash contents of Pacific whiting flesh were reduced by 40.60% and 77.40%, respectively from minced flesh to surimi. Expressible water of all surimi samples was decreased significantly ($p < 0.05$) after processing into surimi (Table 4).

Hunter Color Values

"L" values of all minced flesh samples were increased significantly ($p < 0.05$) after washing steps but, "a" and "b" values were significantly decreased (Table 5). The increasing of "L" value (lightness) and the decreasing of "a" value indicated that most of myoglobin and hemoglobin that responsibly for the red hue of fish meat were removed during washing (Park, 1995). There was no significant difference between "L" and "b" values of surimi fillet and frame from tilapia and grass carp surimi, but grass carp surimi had higher value of blueness. Eid et al. (1991) stated that "L", "a" and "b" values (52.20, 3.00 and 9.6) of unwashed lizardfish mince were changed to L(67.50), a(-2.60) and b(4.80) after washing. Cooked surimi gel of Alaska pollack have higher greenness and lower Yellowness than that of Tilapia and Grass carp gels (Table 6).

Amino Acids

Amino acid profile of surimi fillet and frame from tilapia and grass carp surimi were similar to that of Alaska pollack surimi (Table 7). The overall amino acids score of Alaska pollack surimi is similar to those of land-animal meats (AFDF, 1987).

Minerals

Grass carp surimi had lower level of Ca, Mg, P, K, Zn, Cu, Si, pb, Ba, B, Al and Cr and higher level of Na, Fe, Co, Mn, Mo and Ni than that of surimi from tilapia fillets (Table 8). Frame surimi from Tilapia has the highest level of Fe, Cu, Mo, Ni, Co, Ba, Sr and Cr. Nettleton (1985) reported that Pollack surimi has 143mg/100g sodium, 78mg/100g potassium and 0.26mg/100g iron. The high level of sodiurn content in a surimi sample attributed to adding sodium tripolyphosphate (Nettleton, 1985).

Table 6 - Hunter color values (L, a and b) of surimi gels

Samples	L	a	b
Tilapia surimi	82.44a ±0.69	-1.38b ±0.23	4.63b ±0.10
Tilapia frame surimi	79.03c ±0.20	-1.62a ±0.12	4.42b ±0.15
Grass carp surimi	81.48b ±0.27	1.63a ±0.18	5.97a ±0.24
Alaska pollack surimi*	79.64 ±0.62	-3.84 ±0.03	1.78 ±0.22

* from Kim et al.(1993).

n = 3

abc Means in a column with different letters are different

(P < 0.05)

Table 7 - Amino acid composition of tilapia and grass carp surimi

	Tilapia minced fillets	Tilapia surimi Fillets	Tilapia minced frames	Tilapia surimi frames	Carp minced fillets	Carp Surimi fillets	Alaska ^a pollack
g amino acid/100g protein							
Asp.	10.99	10.75	9.34	11.04	10.99	11.47	11.60
Thr.	4.67	4.78	4.17	5.07	4.79	5.37	4.60
Ser.	3.73	3.35	3.33	3.86	3.99	4.37	5.37
Glu.	17.00	17.31	14.55	18.73	16.09	18.93	18.90
Pro.	3.61	3.00	3.49	3.87	3.91	4.08	3.60
Gly.	4.79	4.92	4.42	4.29	4.17	3.91	4.50
Ala.	5.41	5.70	5.09	5.48	5.48	5.58	6.50
Cys.	0.56	1.08	1.12	0.95	0.68	0.62	1.00
Val.	4.27	4.50	3.80	4.6	4.51	4.94	4.90
Met.	2.88	3.11	2.42	3.47	2.76	3.27	3.10
Iso.	5.02	5.12	4.18	5.69	4.91	5.58	6.20
Leu.	8.38	8.50	7.09	9.35	8.17	9.23	10.60
Tyr.	3.40	3.52	2.83	3.96	3.42	3.72	3.60
Phe.	4.05	3.75	3.36	4.09	3.93	4.13	3.80
His.	2.32	2.22	2.00	2.47	2.28	2.36	2.50
Lys.	9.74	9.74	8.29	10.93	9.62	10.71	11.70
Arg.	6.13	6.21	5.02	6.91	5.67	6.80	7.00
Total	96.96	97.57	84.49	104.77	95.37	105.07	109.30

^a From AFDF, (1987).

Table 8 Macro-elements (Na, Ca, Mg, P, and K mg/100g minced fish) and micro-elements (ppm) content of flesh and surimi processed from tilapia and grass carp

Elements	Tilapia minced fillets	Tilapiasuri mi fillets	Tilapiamin ced frames	Tilapia surimi frames	Carp minced fillets	Carp surimi fillets
Na	47.76	107.37	61.62	106.61	44.12	119.04
Ca	41.31	71.49	49.84	64.44	14.82	25.17
Mg	23.16	13.08	18.68	9.68	21.79	7.65
P	175.82	87.39	133.73	84.89	202.05	84.41
K	342.48	22.81	222.39	9.63	331.79	5.72
Zn	37.68	49.41	40.58	35.20	9.27	5.31
Cu	0.59	0.69	1.80	7.52	0.79	0.47
Fe	17.58	0.00	146.68	55.15	71.62	22.77
Si	8.54	23.10	11.97	17.29	9.77	7.99
CO	0.04	0.00	0.21	0.08	0.07	0.05
Mn	0.00	0.00	0.00	0.00	1.17	0.51
Mo	0.01	0.00	0.31	0.14	0.03	0.02
Ni	2.08	0.56	16.87	5.64	5.43	2.27
Pb	1.22	0.55	0.72	0.26	0.09	0.08
Sr	0.29	0.51	0.31	0.58	0.09	0.56
Ba	0.07	0.19	0.85	0.26	0.03	0.07
B	0.87	0.27	0.45	0.25	2.75	0.04
Al	5.31	3.77	3.51	1.49	4.56	2.30
Cr	3.56	0.94	26.18	9.30	8.87	3.04

Gel Strength

For surimi gels, stress is an indicator for gel strength that affected by the moisture content and heating temperature. True shear strain at failure is used to measure of the protein quality of fish gel and its indicator of gel cohesiveness (NFI,1991). Stress and true shear strain of tilapia and carp surimi were higher than that of Alaska pollack but, tilapia surimi has higher stress and strain than carp surimi (Table 9). Park et al.(1990) stated that stress of tilapia surimi gel prepared in pre-rigor, in-rigor, and post-rigor were 98.16kPa, 81 .51kPa and 70.89kPa, respectively. The strains of that were 2.47, 2.38, and 2.21, respectively. No difference between folding test score for tilapia and carp surimi that received the highest score (AA = 5 extremely elastic) as the same of Alaska pollack surimi. The results agree with the data reported by Somboonyarithia,(1990), surimi processed from tilapia stored in ice ($0\pm 2^{\circ}\text{C}$) for 0, 1 and 4 days has the highest score AA extremely elastic. No significant difference was observed between the expressible water of Tilapia and Carp surimi gels. Gel strength (g.cm) of Tilapia surimi was higher than that of carp and Alaska pollack surimi (Table 10). Gel strength of tilapia (1,061 gxcn) was higher than that data for tilapia (562 gxcn) reported by Somboonyarithia (1990).

CONCLUSIONS

This study showed that the yield of surimi from tilapia and grass carp was lower than Alaska pollack. The yield of surimi from frames was 6.96% of total surimi weight. Most of the water-soluble protein was removed in the first washing cycle. No significant difference between "L", "a" and "b" values of tilapia and grass carp surimi. Tilapia surimi has higher fat and lower moisture content than Carp and Alaska pollack surimi, but carpsurimi has lower protein content than Tilapia and Alaska pollack Grass Carp surimi has lower level of Ca, Mg, P, K and Zn and higher level of Ni, Fe, and Na than Tilapia fillet surimi. Tilapia frame surimi has the highest level of Fe, Ni and Cr. Tilapia and carp surimi produced very elastic gels that have high gel strength. Tilapia and grass carp may be being suitable materials to processed surimi.

REFERENCES

- Alvaerz, C., Couso, I., Solas, M.T., and Tejada, M. 1992. Influence of manufacturing process conditions on gels made from sardine surimi. In "Food Proteins structure and Functionality" K.D. Schwenke and R. Mothes (Eds.), pp. 347-353. VCH Verlagsgesellschaft, Germany.
- AFDF. 1987. Surimi, it's American now. project summary, Alaska Pollack Surimi Industry Development Project. Alaska Fisheries Development Foundation Inc., Anchorage, Alaska.
- AOAC. 1984. Official Methods of Analysis, Association of Official Analytical Chemists, Washington DC.
- Chang-Lee, M.V., Lampila, L.E. and Crawford, D.L. 1990. Yield and composition of surimi from Pacific whiting (*Merluccius productus*) and the effect of various protein additive on gel strength. J. Food Sci. 55: 83-86.
- Douglas-Schwarz, M., and Lee, C. M. 1988. Comparison of the thermostability of Red Hake and Alaska pollack surimi during processing. J. Food Sci. 53: 1347-1351.
- Eid, N., Dashti, B., and Sawaya, W. 1991. Sub-tropical fish by-catch for surimi processing. Lebensmittel-wissenschaft and Technologia. 24: 103-106.
- Hamann, D.D. 1983. Structural failure in solid food. in physical Properties of Food" E. B. Bagley and M. Peleg (Ed.), pp. 351-383, AVI Publishing Co., Westport, C.T.
- Hastings, R.J. 1989. Composition of the properties of gels derived from cod surimi and from unwashed and once-washed cod mince. Int. J. Food Tech. 24: 93-102.
- Hennigar, C. J., Buck, E.M., Hultin, H.O., Peleg, M., and Varelziz, K. 1988. Effect washing and sodium chloride on mechanical properties of fish muscle gels. J. Food Sci. 53: 963-964.
- Holmes, K.L., Noguchi, S.F., and Macdonald, G.A. 1992. The Alaska pollack resource and other species used for surimi. In " Surimi Technology" T.C. Lanier and C.L. Lee (Eds.), pp. 41-76, Marcel Dekker, Inc., N. Y.
- Kim, S-H., Carpenter, J-A., Lanier, T.C., and Wicker, L. 1993. Setting response of Alaska pollack surimi compared with beef myofibrils. J. Food Sci. 58: 531-534.
- Kudo, G., Okada, M., and Miyauchi, D. 1973. Gel-forming capacity of washed and unwashed flesh of some Pacific coast species of fish. Marine Fish Review, 35 (12): 10-15.
- Lee, C.M. 1984. Surimi processing technology. Food Tech. 38 (11): 69-80.
- Lee, C.M., and Chung, K.H. 1989. Analysis of surimi gel properties by compression and penetration tests. J. Text.Stud. 20: 363-377.

- Makinodan, Y., Toyohara, H., and Niwa, E. 1985. Implication of muscle alkaline proteinase in the textural degradation of fish meat gel. *J Food Sci.* 50: 1351- 1355.
- Nettleton, J.A. 1985. Seafood nutrition: facts, issues and marketing of nutrition in fish and shellfish. pp. 11-17, Osprey Books, N. Y.
- NFI 1991. A manual of standard methods for measuring and specifying the properties of surimi. prepared by the technical subcommittee of the surimi and seafoods committee; (T.C. Lanier, K. Hart, and R.E. Martin, eds), National Fisheries Institute, Washington, D C., pp. 6-27.
- Okada, M. 1985. Ingredients on gel texture. In "Engineered seafood including surimi" pp. 507-513, Martin, R.E., and Collette, R.L. (eds.), Noyes Data Corp., Park Ridge, N.J.
- Park, J.W., Korhonen, R.W., and Lanier, T.C. 1990. Effect of rigor mortis on gel-forming properties of surimi and unwashed mince prepared from Tilapia. *J. Food Sci.* 55: 353-355, 360.
- Park, J.W. 1995. Surimi gel colors as affected by moisture content and physical conditions. *J. Food Sci.* 60: 15-18.
- Pigott, G.M. 1986. Surimi : the "high tech" raw materials from minced fish flesh. *Food Rev. Int.* 2 (2): 213-246.
- Reppond, K.D., Edson, S.A., Babbitt, J.K., and Hardy, A. 1987. Observation on the functional properties of U.S. land-processed surimi. *J. Food Sci.* 52:505-506.
- SAS, 1987. SAS/STAT Guide for personal computers, Version 6 ed. SAS Institute, Cary, N.C.
- Somboonyarithi, V. 1990. Effect of iced and frozen storage on quality of surimi processed from Tilapia, (*Tilapia nilotica*). *Asean Food J.* Vol. 5 (4): 158-164.
- Sribhibhadh, A. 1985. Worldwide market prospects for surimi. In "Engineered seafood including surimi" pp. 411-418, R.E. Martin and R.L. Collette, (eds.), Noyes Data Corp., Park Ridge, N. J.
- Weinberg, Z.G., Regenstein, J.M., and Baker, R.C. 1984. Effects of different salts on water retention and heat initiated binding properties of comminuted cod muscle. *J. Food Biochem.* 8: 215-227.
- WU, M.C. 1992. Manufacture of surimi-based products. In " Surimi Technology", T.C. Lanier and C.M. Lee (eds.) pp. 245-272, Marcel Dekker, Inc., N. Y.
- Yean, Y.S 1993. The quality of surimi from Threadfin bream stored on ice for different periods. *Int. J. Food Sci. Tech.* 28: 343-346.

Table 9 - Gel strength of surimi processed from tilapia and grass carp

Samples	Shear stress kPa	True shear strain	Rigidity KPa	Folding test	Expressible water%
Surimi fillet*	86.81a ±8.93	2.69a ±0.13	32.33b ±2.83	AA=5	6.1 lb ±0.67
Washed mince*	57.20b ±10.73	1.88d ±0.23	30.15b ±2.94	AA=5	6.05b ±1.02
Surimi frame*	84.59a ±12.76	2.21c ±0.22	38.13a ±1.94	AA=5	5.95b ±0.54
Carp surimi	52.32b ±4.53	2.47b ±0.15	21.299d ±2.51	AA = 5	6.33b ±0.93
Washed mince	40.395c ±3.89	1.67e ±0.08	24.23c ±1.79	B = 3	9.50a ±0.96
Alaska pollack surimi ^a	49.40 ±11.5	2.19 ±0.31	22.90 ±5.60	AA=5	NA

a from Reppond, et al., (1987).

n = 6

* Samples prepared from tilapia.

abcde Means in a column with different letters are different(p < 0.05).

Table 10 - Gel strength (gxcn) of tiapia and grass carp surimi

Samples	Breaking Force(g)	Breaking distance (cm)	Gel strength (gxcn)
Surimi fillet*	683.71a ±82.37	1.55a ±0.17	1061.46a ±216.87
Washed mince*	380.00b ±22.17	1.04c ±0.05	395.20c ±31.03
Frame surimi*	625.00a ±71.89	1.38b ±0.16	862.50b ±196.67
Carp surimi	306.43c ±19.12	1.07c ±0.08	327.73c ±42.11
Washed mince	211.00d ±12.49	0.71d ±0.05	149.81d ±16.46
Alaska pollack surimi ^a	440.00 ±87.00	1.39 ±0.14	617.00 ±153.00

a from Reppond, et al., (1987).

n = 6

* Samples prepared from tilapia.

abcd Means in a column with different letters are different(p < 0.05).