#### AN ABSTRACT OF THE THESIS OF

<u>Steven Eldon Berntsen</u> for the degree of <u>Master of Science</u> in <u>Food Science and Technology</u> presented on <u>July 28, 1987</u>.

Title: <u>Development of a Restructured Seafood Product from Squid</u> (Loligo opalescens).

The influence of protein adjuncts and variations in pH on the cook-cool loss, moisture content, and texture of squid gels was investigated. Break force ( $P \ge .025$ ), deformation to break ( $P \ge .01$ ) and cook-cool loss ( $P \ge .001$ ) decreased as the pH of squid gels was adjusted from 6.4 to 8.3 with sodium carbonate. The moisture content of gels increased ( $P \ge .01$ ) as the pH was elevated. Break force ( $P \ge .001$ ) and deformation distance to break ( $P \ge .005$ ) were inversely correlated to gel moisture content.

Protein adjuncts (2 %) had a significant effect on cook-cool loss, break force and moisture content ( $P \ge .001$ , .026, and .018, respectively) of squid gels. Egg white produced an improved water-holding capacity over soy protein isolate and sodium caseinate. The mean cook-cool loss from gels containing egg white was 5.29  $\pm$  0.66 % which was significantly (P = .05) lower than that of gels containing soy protein isolate (12.41  $\pm$  0.17 %), no protein adjunct (16.65  $\pm$  3.82 %) and sodium caseinate (19.75  $\pm$ 2.42 %). Gels containing sodium caseinate had a significantly (p>.05) lower moisture content and higher cook-cool loss (P=.05) than gels containing egg white or soy protein isolate.

The break force of gels containing sodium caseinate could not be measured because the gels possessed little fracturability when compressed. The break-force of gels containing soy protein isolate or egg white were equal (P=.05), but each was less (P=.05) than observed for control gels.

Holding minced squid gel sols at  $4^{\circ}$ C for 4 and 24 hours before forming and heat-setting into gels, had a significant effect on the force required to break gels (P $\geq$ .001) and expressible moisture (P $\geq$ .006). Control squid gels and gels containing soy protein isolate that were formed after holding for 24 hours required a lower force (P=.05) to break gels than similar gels formed after holding for 4 hours. Holding time did not alter (P $\leq$ .05) moisture content, cook-cool loss or deformation at break. Reduced gel strengths observed for gels held for 24 hours prior to heat-setting was related to protease activity, loss of reactive sulfhydryl functions and/or cold temperature setting.

A restructured seafood product was fabricated from mantle and tentacle muscle of squid (Loligo opalescens). Whole squid yielded  $37.5 \pm 1.4$  % edible mantle and  $15.3 \pm 1.0$  % tentacle meat; a total yield of  $52.2 \pm 2.4$  % based upon round weight. A restructured product fabricated from heat-set fibers (60 %) and squid sol (40 %) yielded  $33.4 \pm 1.0$  % based upon round weight. A consumer preference panel found no (P $\leq$ .05) differences in the product containing different protein adjuncts and judged the product to be, at least, slightly desirable (score of 6.0 on a 9 point scale). Firmness was the only sensory attribute that a trained panel found to be influenced ( $P \ge .05$ ) by the incorporation of protein adjuncts. The product containing sodium caseinate was judged to be less firm (P = .05) than products incorporating either egg white or soy protein isolate. Trained panel scores for firmness were consistent with measurements for break force.

# Development of a Restructured Seafood Product

from Squid (Loligo Opalescens)

by

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INTRODUCTION	1
LITERATURE REVIEW	5
Biology and Life History of Market Squid (Loligo opalescens)	5
Fishing Methods and Gear	7
Japan	7
Eastern U.S.	7 7
California	- Y
Overview of the 1985 Oregon Squid Harvest	8
The oregon squid Situation in 1986 and 1987	9
On-Doard-Mandling	11
Squid Processing	11
Thermal Machanical Skinning	10
Chemical Machanical Skinning	14
Enguno Mochenical Skinning	14
Enzyme-Mechanical Skinning Microstructure of Squid Mussle	10
Composition of L onelessons	10
Podu Componente	10
Body components Drawingto Correction	10
Characterization of Proteins	10
Characterization of Proteins	10
Frotein Amino Actu composicion	79
Non-protoin nitragen	20
Non-protein nitrogen	20
Caunepsins Stopage Stobility of Squid	22
Storage Stability of Squid Restructured Sectord Products	24
Restructureu Sealoou Products	20
Solubilization of Actomyosin	20
Get transformations	29
Texture and Ingredients	31
Flavoningo	30
Flavorings Postmucturing Techniques	30
Restructuring lechniques	31
MATERIALS AND METHODS	39
Raw Materials	39
Squid Processing	39
Cleaning and Dewatering	39
Experimental Product Preparation	40
Restructuring Squid Product	41
Evaluation Procedures	43
Cooking and Cool Loss	43
Moisture Determination	43
Determination of pH	43
Proximate Composition	44
Instron Measurements	44
Sensory Evaluation	45
Statistical Analysis	47

Table of Contents (Continued)

RESULTS AND DISCUSSION	49
Adjustment of Gel pH	49
Effect of Sodium Carbonate on Gel pH	49
Effect of Gel pH on Cook-Cool Loss and Moisture Content	50
Effect of Gel pH on Break Force and Deformation at Break	51
Protein Adjunct	53
Effect of Holding Time	53
Effect of Protein Adjuncts	57
Preparation and Evaluation of a Restructured Squid Product	66
Preparation of Restructured Squid Product	66
Composition of Raw Squid and Restructured Squid Product	67
Sensory Evaluation of Restructured Squid Product	69
SUMMARY AND CONCLUSIONS	72
BIBLIOGRAPHY	76

LIST OF TABLES

able	F	age
1	Composition of edible portion of raw squid ( <u>Loliginidae</u> spp.)	17
2	Comparison of the essential amino acid content in the protein of the edible meat of squid and fish	19
3	Sensory attributes and selection of reference samples	46
4	Effect of varying levels of sodium carbonate on the moisture content, cook-cool loss, break force and deformation at break of squid gels	51
5	Break force (g) of squid gels containing different protein adjuncts formed and heat-set after varying holding times at 4 <sup>0</sup> C	61
6	Expressible moisture of squid gels containing different protein adjuncts formed and heat-set after varying holding times at 4°C	62
7	Moisture content (%) of squid gels containing different protein adjuncts formed and heat-set after varying holding times at 4°C	63
8	Cook-cool loss of squid gels containing different protein adjuncts formed and heat-set after varying holding times at 4 <sup>0</sup> C	64
9	Deformation of squid gels containing different protein adjuncts formed and heat-set after varying holding times at 4 <sup>°</sup> C	65
10	Processing yields for restructured squid product	67
11	Composition of raw edible squid muscle and restructured squid product	68
12	Mean consumer preference scores for various sensory attributes of a restructured squid product containing different protein adjuncts.	69
13	Mean trained panel magnitude estimation scores for various sensory attributes of restructured squid products containing different protein adjuncts	70

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Table

## DEVELOPMENT OF A RESTRUCTURED SEAFOOD PRODUCT FROM SQUID (LOLIGO OPALESCENS)

#### INTRODUCTION

Squid has long been a traditional food in Oriental and Mediterranean cultures. Today it is a food staple in 17 countries and is prepared in a wide variety of ways (Kruezer, 1986). Cultures that have traditionally rejected squid from the diet are now beginning to appreciate its culinary significance.

The four major squid consuming countries of the world are Japan, Spain, Korea, and Italy. Japan consumes by far the most, at least half the total world catch (Kreuzer, 1986).

As the world population continues to grow, so does the demand for squid. Approximately 1.2 million metric tons of squid were harvested worldwide in 1983 by 55 nations (Kreuzer, 1986).

Some 270 species of squid exist throughout the oceans of the world. Five species are of major commercial importance. <u>Todaradus</u> <u>pacificus</u> or Japanese squid is harvested in greatest quantity, 32% of the world squid catch in 1980. <u>Illex illecebrosus</u> (summer or short-finned squid), <u>Notodarus sloani</u> (Tasmanian squid), <u>Loligo</u> <u>peali</u> (winter or long-finned squid), and <u>Loligo opalescens</u> (market or California squid) comprised 10, 4, 2 and 1 percent of the total 1980 harvest, respectively (Veasy and Blaxall, 1983).

Three species are harvested off North America. <u>Illex</u> <u>illecebrosus</u> occur nearshore off Newfoundland and in deeper waters as far south as Florida. <u>Loligo peali</u> are strictly a coastal species ranging from the continental shelf to inshore waters from Massachusetts to Florida and in the Gulf of Mexico. On the Pacific coast, <u>Loligo opalescens</u> are found near shore from Baja California, Mexico to British Columbia, Canada. One other species, <u>Dosidicus</u> <u>gigas</u> or jumbo squid, is harvested in Central American waters and is occasionally found off southern California, but, not frequently enough to support a fishery.

The United States harvested 1.4% of the world squid catch in 1980. An average of only 5 to 15% of the annual U.S. squid catch was consumed in the domestic market each year from 1976 to 1981 (Veasy and Blaxall, 1983). Most of the squid was either exported as frozen or canned product.

Annual California squid landings averaged around 15,500 metric tons during the years 1976 to 1981. Annual squid landings on the entire Atlantic coast of the United States during those years averaged 3,500 metric tons.

The California squid harvest dropped dramatically in 1983 to 2,200 metric tons and again in 1984 to only 565 metric tons. The unfavorable ocean conditions associated with the El Nino phenomenon that occurred in 1982, is believed to have been responsible for this decline in landings. The California squid fishery showed signs of recovery in 1985 with an annual harvest of 9,900 metric tons, however, it dropped again in 1986 to 5,000 metric tons (Starr and McCrae, 1986).

In contrast to the declining annual squid landings in California, total landings on the Atlantic coast of the U.S. have steadily increased since 1980 to 14,000 metric tons in 1984.

The Oregon squid fishery began in 1982 and expanded each season through 1985. Landings totaled 795 metric tons in 1985, double the 1984 harvest. Oregon squid landings plummeted to only 12 metric tons in 1986 primarily because fishing efforts were directed toward shrimp (<u>Pandalus jordani</u>), a more profitable species (Starr and McCrae, 1986). The squid fishery in Oregon was practically non-existent again in 1987 due to the same economic situation present the previous year.

Practically the entire 1985 Oregon catch was frozen whole and exported to foreign markets (primarily Japan, followed by Spain and Italy). Sixty to seventy percent was processed and packed as food grade product, the remainder was marketed as bait (Starr, 1985a).

Squid is gaining wider acceptance in the U.S. and can be found more and more frequently in restaurants and fish markets. To meet the ever increasing demand the United States imports much squid from abroad, roughly 3,800 metric tons in 1982 (Rodriquez, 1983). Much of the imported squid is in the form of cleaned tubes and tentacles and is reasonably priced because labor costs are low in those foreign countries.

Processing squid by hand into an edible and marketable product is very labor intensive. The recently developed eviscerating and skinning machines are capable of producing 500 pounds per hour of cleaned product (skin-free mantles and skin-on tentacles) and yield 50 to 55 percent edible muscle from whole market squid (Squid Machine Corp., 1983). This technology will greatly improve the efficiency of processing squid into marketable products.

The tentacles are poorly accepted by the average North American consumer largely due to the unappetizing appearance and mouth feel. This is unfortunate because the tentacles are a palatable, high quality protein source comprising a sizable portion (30%) of the total yield of edible muscle from whole squid.

Restructuring the mantle and tentacle muscle into a form with textural properties similar to comminuted meat products, such as sausage, may improve the acceptance and marketability of squid in the United States. Textural modification of surimi products by the addition of starches and protein from various sources (soy, milk, egg white, and wheat) has been reviewed by Lee (1986). It may be possible to impart a meaty texture to restructured squid products with the aid of starch and protein adjuncts.

The squid resource off Oregon has the potential to support a sizable fishery. The development of a domestic market would increase the dock-side value for squid and in turn generate more revenue to the local fishery. Processing and distribution of squid products would generate more personal earned income and benefit Oregon's economy.

The objective of this investigation is to improve the acceptance of squid (Loligo opalescens) by restructuring the mantle and tentacle muscle into a form similar to comminuted meat products. The influence of pH, various protein adjuncts (egg white, soy protein isolate, and casein), and refrigerated storage of squid sols before forming and heat-setting, on the cook-cool loss, expressible moisture, and textural properties of squid gels were investigated.

#### LITERATURE REVIEW

## Biology and Life History of Market Squid (Loligo opalescens)

Squid are taxonomically classified as a member of the phylum Mollusca, group Cephalopod and order Teuthoidea. Squid are closely related to oysters and clams. <u>Loligo</u> and <u>Illex</u>, the most important species, are differentiated into separate suborders; Myopsida and Oegopsida, respectively. Unlike the oceanic habitat of <u>Illex</u> species, <u>Loligo</u> seldom live beyond the continental shelf (Berk, 1974).

Loligo opalescens are abundant off the Pacific coast of North America from the southern tip of Baja California  $,22^{\circ}$ N, to British Columbia  $,50^{\circ}$ N (Jefferts, 1983). During the various developmental stages of its life, <u>L. opalescens</u> live at all depths on the continental shelf and even beyond the shelf to depths of 460 meters. Having a strong preference for water with a higher salinity, this species is absent in estuaries and in the vicinity of large volume, fresh water effluents such as that occurring off the mouth of the Columbia River. They are, on the average, smaller than the two commercial squid species (<u>Illex illecebrosus</u> and <u>Loligo pealei</u>) that occur off the Atlantic coast (Fields, 1965). Males, with an average mantle length of 14 cm and weight of 70 g are generally larger than females with an average mantle length of 14 cm and weight of 50 g.

<u>L. opalescens</u> aggregate during feeding and spawning. Feeding schools are less dense and more easily dispersed by fishing activity than spawning schools. Krill (<u>Euphausia pacifica</u> and

<u>Thysanoessa spinifera</u>) comprise the bulk of their diet. Finfish, gastropods, polychaete worms, planktonic crustaceans, and other cephalopods, including their own species, supply the remainer of their diet. Market squid are a vital link at the lower end of the marine food chain and are a principal food item for at least 22 species of fish, 13 species of birds, and most marine mammals (Hixon, 1983).

L. opalescens move inshore to spawn in water 5-40 meters deep with a maximum spawning activity at 15 meters (Jefferts, 1983). This species prefers sheltered bays with either a sand or mud bottom. Reproduction has been observed year round with certain periods of peak activity (Jefferts, 1983). The spawning period off the Oregon coast extends from April to July with the highest activity occurring in May and June (Jefferts, 1983). Squid are harvested in Oregon during this period because they aggregate in large spawning masses and can be caught very efficiently.

L. opalescens are terminal spawners. They die soon after spawning at one to two years of age. The majority spawn and die between 14 and 22 months of age (Spratt, 1978). Squid undergo drastic physiological changes during this period. Fully mature reproductive organs comprise 25 to 50 % of the total body weight of the female (Fields, 1965). Feeding is minimal or nonexistent and physical deterioration proceeds rapidly. Total body weight declines by over 50 % and mantle tissue thickness decreases 24 % in males and 42 % in females (Fields, 1965). The squid are severely battered; arms are broken, and skin is torn (McGowan, 1954).

### Japan

The many species of squid that abound throughout the oceans of the world are harvested by almost as many different fishing methods. Japan harvests more squid than any other country in the world. Ninety percent of the Japanese squid harvest is taken by jigging methods (Amos, 1983). Drift gillnets, and purse seines are also used. The best quality squid on the Japanese market are jig-caught. The percentage of squid landed by jigging is declining as Japan resorts to foreign squid resources to meet their growing demand. The major portion of the squid that has been harvested by Japan off the coasts of North America, Argentina, and Africa was taken by trawlers (Court, 1980).

## Eastern U.S.

The squid resource off the East Coast of the U.S. is largely exploited by foreign countries (Japan, Spain, Italy, Poland, West Germany and the USSR) either by foreign trawlers or cooperative joint fisheries. Of the 136,000 metric tons of squid that were harvested off the East Coast in 1983, only 10 % (13,600 metric tons) constituted domestic landings and most of this was incidental to trawling for other species (Rathjen, 1983).

## California

The California squid fishery actually consists of two factions, the southern California fishery and the Monterey fishery

(Recksieck and Frey, 1978; Kato, 1983; Dewees and Price, 1981). The southern fishery occurs between December and April near the California Channel Islands. Purse seines are used extensively. Squid are also attracted with lights at night and brailed or pumped aboard.

In Monterey Bay, squid are taken between April and November exclusively by lampara nets. This fishery is highly regulated. The use of purse seines was outlawed in 1953 because the lead line was thought to be disruptive to the squid eggs rooted to the bottom. The Monterey fishing industry imposed regulations in 1959, prohibiting the use of lights to attract squid. Fishermen felt they needed to protect their markets from processors who could attract squid with lights at their piers and harvest them with dip nets. They also contended that light-attracted squid were generally spawned out and dying and that the quality of the meat was poor. This is verified by the different dock prices of squid for the two fisheries. San Pedro light-attracted squid are mostly used for canning and receive half the dock price of Monterey lampara-caught squid which are considered higher quality and marketed as fresh and frozen (Rodriquez, 1983).

# Overview of the 1985 Oregon Squid Harvest

The 1985 squid harvest in Oregon occurred over a six week period in April and May (Starr, 1985b). Sixteen vessels made 65 landings, averaging 26,900 lb. per landing. Six vessels accounted for 77 % of the landings. The purse seine was the primary fishing gear used. Fifty eight percent of the 65 landings came from

vessels with purse seines, 22 % from shrimp trawls, and 20 % from lampara nets. Sixty six percent of the 795 metric tons of squid landed in Oregon during the 1985 season was harvested with purse seines, 19 % with shrimp trawls, and 16 % with lampara.

Ex-vessel price began at \$500-600 per ton at the start of the season and dropped to \$200-300 per ton toward the end. Average ex-vessel price for the 1985 season was \$350 per ton.

# The Oregon Squid Situation in 1986 and 1987

Economics played a major hand in shaping the Oregon squid fishery in 1986 and 1987. Processors in 1986 predicted they could sell all the food grade squid that fishermen could deliver. There was a strong world market for Loligo species that year because the harvest of <u>Loligo vulgaris</u> around the Falkland Islands off Argentina was extremely poor (Starr and McCrae, 1986).

The Oregon squid fleet, in March, consisted of 7 boats with experimental trawl permits and 4 boats equipped with purse seines. Efforts to locate schools of squid had failed. The shrimp season opened in April with large early-season landings averaging 6.8 metric tons, and an ex-vessel price of at least \$1100/MT (\$0.50/lb). Most of the squid boats immediately converted to shrimping (Starr and McCrae, 1986).

Oregon squid landings in 1986 totalled 12 metric tons. Most of the squid was caught just south of Newport by two boats using purse seines during a one week period in May. The boats made three landings averaging nearly 4 metric tons per trip and

received an ex-vessel price of 220/MT (0.10/lb) (Starr and McCrae, 1986).

When the shrimp season opened in 1987, prospects looked even better than the year before. Early-season landings were larger and the ex-vessel price was \$1540/MT (\$0.70/lb) or more. Consequently few, if any, boats in Oregon fished for squid in 1987.

#### On-board Handling

In California and Oregon, squid are neither refrigerated nor iced on board the vessels as fishing grounds are close to port. Most fishing occurs at night and the squid are delivered and processed the following morning. Squid are unloaded and transferred to the dock with fish pumps from flooded vessel holds.

Special precautions must be taken by fishermen to prepare their vessels for stowing squid. Squid is a very dangerous cargo because of their slimy and watery nature. The squid must be securely contained within the bins by strong binboards to prevent the load from shifting in heavier seas. All possible gaps in the fish hold floors and walls should be covered with 1/2 inch mesh wire to prevent the squid from slipping into other boat compartments. To facilitate good drainage, standpipes studded with 1/2 inch holes should be placed vertically along the sides of the bins.

On the East Coast of the U.S., the smaller boats that are not equipped with freezers often bulk-ice squid during extended trips (Klos, 1983). Squid are crushed, tentacles and ink sacks are broken and skin is torn by the sharp ice. The highest quality squid is taken with traps, iced onboard with crushed ice (smooth edges) and transferred to barrels containing chilled seawater at the dock. This system cools the product quickly, washes out the skin pigments and ink, and eliminates crushing and ice damage because the squid are floated. Refrigerated sea water holding and non-contact icing are proven, superior methods for handling squid (Learson and Ampola, 1981), but have yet to be adopted by the West Coast fishery.

Squid is an extremely perishable seafood in the fresh state, however, its frozen storage stability is unique. The period between capture and freezing is extremely critical for quality assurance. Optimal squid handling practices were observed onboard a Japanese processing ship by Lund (1981) who reported that squid were frozen on board within 6 hours of capture. The Japanese froze squid in the first natural state of color, with spots, and when the squid turned white it was considered to be in the second stage of deterioration. Lund (1981) recorded the following recommended procedures for freezing at sea to insure a top quality product: (a) wash freshly caught squid; (b) sort to size and pre-chill in clean ice water to  $2-7^{\circ}C$ ; (c) pack in trays and freeze at  $-40^{\circ}C$  to an internal temperature of  $-20^{\circ}C$ ; (d) glaze and package in poly-lined corrugated boxes and (e) store at  $-20^{\circ}C$ .

## Squid Processing

The processing and marketing of squid in California is more diverse than in Oregon. Three quarters of the annual catch is marketed as frozen whole squid (Dewees and Price, 1981). Most of

this (80 %) is exported, primarily to Japan, and southern Europe. The remaining 15-20 % of the frozen whole squid is sold as bait. Squid are also thermal processed in the round in one pound cans for export. A small portion of the catch, 2-3 %, is marketed fresh through retail and food service establishments. Processed squid include the following frozen products: cleaned and skinned mantles, tentacles, breaded mantles and tentacles, stuffed squid and squid burgers.

Despite its low price, 1.52-2.18/kg, whole squid (in the round) are not readily accepted by the consumer due to their unappetizing appearance and inconvenience. Processing squid by hand adds 3.30-4.40/kg to the final price. The retail cost for manually cleaned tentacles and mantles ranges from 4.82-6.58/kg (Brown and Singh, 1980). Brown and Singh (1980) have developed a machine that eviscerates and skins <u>L. opalescens</u>. Squid cleaning machines presently in use can run 120 squid per minute at 90 % efficiency and yield 55 % cleaned product at a cost of 0.44-0.88/kg (Squid Machine Corp., 1983).

In contrast, practically the entire 1985 Oregon catch was frozen whole and exported to foreign markets. Sixty to seventy per cent was processed and packed as food grade product, the remainder was marketed as bait. Food grade squid was washed with fresh water to leach ink and skin pigments, rendering a white product. During the wash, squid gained 5-10 % of their weight as water. They were size graded, hand packed into 5 lb. cartons and frozen.

Most squid products require removal of the skin to prevent discoloration, off-flavors and odors (Buisson et al., 1985). Manual skinning is extremely time consuming. Mechanization of the skin removal step would greatly increase the efficiency of the entire processing operation. Many alternatives to manual skinning have been devised for mantles but little work has been done with the tentacles. Of the three skin removal methods that can be applied to tentacles, only enzyme-mechanical skinning has potential at this time.

# Mechanical Skinning:

Learson and Ampola (1981) found that the Jensen Type A fillet skinner, which uses an endless knife blade, performed well at removing the skin from split mantles, but tubes required two passes. A machine made by Townsend Machinery Corporation designed for removal of the membranes from beef liver proved to be excellent for skinning tubes and splitting mantles. Although this machine functioned well with its rotating drum and stationary knife blade, it needed to be redesigned for automatic feeding and increased output. The same researchers tested a cold contact skinner (TRIO) for removing the skin from squid mantles. Mantle skin was flash frozen onto a refrigerated drum and stripped from the mantle tissue when the tissue was pulled off the drum. This method worked well with split mantles but multiple passes were required for tubes.

### Thermal-Mechanical Skinning

Thermal skinning can only be used when some denaturation in the final product is acceptable (Buisson et al., 1985). In Japan, squid used for drying and canning are commonly skinned by heating for 5 seconds at  $80-100^{\circ}$ C or for 15 minutes at  $60-65^{\circ}$ C to denature the skin and loosen it followed by stirring to remove the skin with abrasion (Wilson and Gorham, 1982). Brezeski (1981) found that a quick blanch, in a brine solution, under agitation was satisfactory for skin removal and resulted in little protein loss. Learson and Ampola (1981) used an "oscillating-type washing machine agitator" fitted with expanded metal mesh on the vanes and periphery of the tub to remove the skin from squid mantles that were previously subjected to various thermal treatments. They sought a treatment that optimized skin removal and minimized thermal damage to tissue proteins. The results indicated that a combination of blanching and agitation was effective in removing skin, however, some denaturation of the flesh could not be avoided.

## Chemical-Mechanical Skinning

Learson and Ampola (1981) used either hot or cold solutions of sodium hydroxide or citric acid to loosen the skin from mantles followed by neutralization and skin removal by strong water sprays. The flesh was unacceptably damaged by the high concentration (>2 %) of sodium hydroxide that was required to loosen the skin. A more acceptable treatment that did not

appreciably change the flesh consisted of dipping the mantles in 0.5-1.5 % citric acid for 1-2 minutes at  $38-60^{\circ}C$ .

#### Enzyme-Mechanical Skinning

Immersion of the mantles for 1-2 hours in dilute solutions of ficin, pancreatin and pepsin has been found to be effective for removing the skin, however, the mantle flesh was severely damaged (Learson and Ampola, 1981). Papain was not effective.

Buisson et al. (1985) described a Norwegian patent (Raa, 1983) on an enzyme method for skinning squid that was carried out at temperatures below  $10^{\circ}$ C. The enzyme called DiZYM is extracted from squid and specifically denatured the skin without affecting the muscle tissue. The method is currently being used by Norwegian processors at a cost of six U.S. cents per kg. product.

## Microstructure of Squid Muscle

The major portion of squid mantle consists of muscular tissue sandwiched between two tunics of connective tissue. An external layer of skin surrounds the outer tunic and beneath the inner tunic lies an internal layer of skin lining the muscle cavity (Otwell and Hamann, 1979). The muscular tissue consists of alternating bands of circular and radial, obliquely striated muscle fibers interspersed with connective tissue fibers (Ward and Wainwright, 1972). Most of the connective tissue in squid is located within the fibrous tunics, however, a minor portion comprises the net-like meshwork of small extracellular fibers that are interspersed throughout the muscular tissue (Stanley and Smith, 1984).

### <u>Composition of L. opalescens</u>

### Body Components

Approximately 50 % of whole squid (<u>L. opalescens</u>) is utilized as food in the United States. About 66 % of the edible portion is composed of mantle with the fins (eviscerated and skinned) and the remaining 33 % is tentacles (skin-on) (Hartzell, 1983). The yield of edible flesh is relatively low compared to the yields reported for other North American squid species, i.e., 80 % for <u>Loligo</u> <u>pealei</u> and 63 % for <u>Illex illecebrosus</u> (Gora et al., 1973). Since <u>L. opalescens</u> are harvested during the spawning season, the females are laden with roe which significantly reduces the yield of edible flesh from the whole squid. The roe can be a valuable commodity in the Orient where it is considered a delicacy. Although squid is not universally consumed, uses for all parts of the squid body (food and non-food) have been discovered and implemented by consumers in various countries. A complete summary of these are given by Buisson et al. (1985).

# Proximate Composition

The composition of squid is characterized by its high moisture and protein and low fat content. The average proximate composition of squid (<u>Loliginidae</u> spp.) reported by different investigators is listed in Table 1.

	Percent (wet wt.)				Energy	
	Protein	Fat	Moisture	Ash	Carbohydrate	(Cal/ 100 g)
Mean	15.3	1.0	79.3	1.8	3.0	89
S.D. <sup>2</sup>	1.1	0.2	1.6	0.3		
Range	11.9-18.4	0.5 - 1.4	74.2-84.0	1.0-3.1		80-98
References <sup>3</sup>	6	6	6	7	1	2

Table 1. Composition of edible portion of raw squid (<u>Loliginidae</u> spp.)<sup>1</sup>

From Sidwell et al., 1974

2 Standard deviation

Number of references cited for determining means and range

## Characterization of Proteins

Using a modification of the method of Dyer et al. (1950), Matsumoto (1958b) estimated the protein composition of squid to be similar to that of the white meat of fish with the exception of stroma components. The following major protein contents were determined: myosins (myosin, actin, and actinomyosin), 77-85 %; non-myosins (myogen, myoalbumin, globulin X), 12-20 %; stroma (collagen, elastin), 2-3 %.

The ratio of myosin to actin in squid meat is about 1:1 compared to 4 or 5:1 for fish (Gora et al., 1973). Non-myosin protein accounts for 20 % of the total muscle proteins in squid. An unusually large portion (50-55 %) of the total muscle proteins of squid are water soluble (Matsumoto, 1958b). The non-myosin proteins in fish flesh comprise the entire portion of the water soluble protein fraction. This is not the case for squid where non-myosin proteins comprise only 20 to 33 % of the water soluble protein fraction (Migita & Matsumoto, 1954). The streaming birefringence observed for aqueous extracts of squid has been identified as myosin proper and actomyosin. Hence, squid muscle is unique in that a portion of the contractile proteins are water soluble. These proteins have been identified in the flesh of other molluscs and have been labeled M-actomyosin by Matsumoto (1958a). M-actomyosin account for 30 % of the water soluble proteins of squid and 1-9 % of the total muscle proteins (Matsumoto, 1958a).

Invertebrate muscle (such as squid, shrimp, etc.) contains a characteristic muscle protein, paramyosin, which is not present in vertebrate muscles (Sano et al, 1986). Paramyosin comprises a significant portion of the myofibrillar proteins in marine invertebrate muscle, 14 % in squid obliquely striated muscle (Horie et al., 1975), 3 % in scallop striated muscle (Szent-Gyorgyi et al., 1973), 19 % in oyster striated muscle and 38 % in oyster smooth muscle (Szent-Gyorgyi et al., 1971). Noguchi (1979) speculated that the presence of paramyosin was responsible for the highly elastic and cohesive texture of marine meat gel products made from invertebrate meat.

The collagen content of squid meat is higher than for osseous fish. The meat of squid mantles contains 2.5-3 % collagen compared to 0.5-1.2 % in osseous fish. Although these values vary in the annual cycle, it can be assumed that on the average the meat of squid mantles contains about 3 times more collagen than fish meat (Kolakowski & Gajowiecki, 1973). Stanley and Hultin (1981) reported squid mantle to contain greater than 1.5 % collagen.

## Protein Amino Acid Composition

The protein composition of squid is unique from that of osseous fish (Table 2). No differences in the compositions of amino acids among different species of squid has been observed. The major amino acids (accounting for 50-54 % of the total) occurring in skinned mantle flesh are, in decreasing order of abundance: glutamic acid, aspartic acid, arginine, lysine, and leucine (Hayashi & Takagi, 1979). The essential amino acid content of the edible portion of squid is similar to that of fish with the exception that squid contains a higher level of tryptophan and a lower level of valine.

Amino Acid	G amino acid/1	.00 g protein	FAO standard (1957)	Coefficient of limiting amino acid as compared with FAO standard	
	Edible meat of squid	Fish meat (average)		Squid meat protein	Fish meat protein
Lysine	9.54	9.46	4.2	227.1	225.2
Valine	4.55	5.78	4.2	108.3	132.9
Threonine	4.82	4.94	2.8	172.1	176.4
Tryptophan	1.68	1.10	1.4	120.0	78.6
Methionine	3.08	2.97	2.2	140.0	135.0
Tyrosine	3.18	3.47	2.8	113.5	123.9
Cystine Leucine and	1.35	1.25	2.0	67.5	62.5
isoleucine	13.28	13.94	10.0	147.5	154.9
Total	46.18	46.87	31.4		

Table 2. Comparison of the essential amino acid content in the protein of the edible meat of squid and fish.

<sup>1</sup> From Kolakowski and Gajowiecki, 1973

#### Lipid content

Hayashi and Takagi (1979) reported no differences in the compositions of fatty acids among four species of squid. The predominant fatty acid components of skin-less mantle flesh were: 22:6 (43.0-51.3 %), 16:0 (16.0-26.6 %), and 20:5 (11.6-19.4 %). Omega-3 fatty acids (22:6 and 20:5) comprised 57.8-70.7 % of the total lipid content. Jangaard and Ackman (1965) reported 53 % of the total lipid content of <u>I. illecebrosus</u> to be omega-3 fatty acids (22:6, 37.1 %; 20:5, 15.8 %). The proportion of the total lipids in squid that are omega-3 fatty acids is relatively high compared to the 10-20 % portions occurring in most other marine species. (Gruger et al., 1964).

#### Non-protein nitrogen

The review of Takahashi (1965) lists the many compounds that have been identified in non-protein extracts of squid muscle. These include free amino acids, TMAO, betaine, taurine, guanine, carnitine, adenine, xanthine, and hypoxanthine.

Konosu et al. (1958) identified 17 different amino acids comprising a quarter of the nonprotein nitrogen in squid muscle. The predominant amino acids, in order of decreasing abundance, were found to be histidine, arginine, glycine, and alanine.

Many investigators have reported high levels of trimethylamine oxide (TMAO) and a very active TMAO-ase in squid. Squid mantle muscle contains 100-200 mg % TMAO-N (Shimudu and Takeda, 1952; Konosu et al., 1958; Endo et al., 1962; Takagi et al., 1967; Harada et al., 1968). These levels are comparable to the large amounts present in elasmobranchs. Members of this group (sharks and rays) contain the highest levels of TMAO of all marine species (Dyer, 1952; Harada, 1975). Harada (1975) reported 10.7-11.1 mM/100g of TMAO in the mantles of <u>L. opalescens</u> and found the average level of TMAO in the mantles of 14 species of squid to be 200 mg % TMAO-N (14.3 mM/100g). As a comparison to Gadoid species, Dyer (1952) determined the levels of TMAO in squid (<u>L. opalescens</u>) and cod (<u>Gadus morhua</u>) to be 150-156 mg % TMAO-N (11.1 mM/100 g) and 95 mg % TMAO-N (6.8 mM/100 g), respectively.

Stanley and Hultin (1984b) analyzed frozen squid mantles of three different species (<u>I. illecebrosus</u>, <u>L. pealei</u>, and <u>L.</u> <u>opalescens</u>) for dimethylamine (DMA) content and found 1.34, 0.075, and 0.020 mM/100 g, respectively. The rate of DMA formation in squid was comparable to that found by Lundstrum et al. (1982) for red hake, a gadoid species noted for its TMAO-ase activity (Parkin & Hultin, 1982; Castell et al., 1971). Freezing squid tissue is not essential for the initiation of TMAO-ase activity and the formation of DMA (Stanley and Hultin, 1984b; Parkin and Hultin, 1982). No difference in the rates of DMA formation in frozen and fresh mantle muscle of <u>I. illecebrosus</u> has been observed (Stanley and Hultin, 1984b). Stanley and Hultin (1982) indicated that this may be a consequence of the active autolysis that takes place in post-mortem squid muscle (Leblanc and Gill, 1982; Stanley and Hultin, 1984a).

### Cathepsins:

The proteolytic activity found in the skeletal-muscle extracts of porcine, bovine, chicken, and fish has been attributed to a group of hydrolytic enzymes known as cathepsins, which are localized within the lysosomes of the tissue (Reddi et al., 1972). Siebert (1958) observed that the cathepsin activity of fish muscle was ten times greater than that of mammalian tissue. Makinodan et al. (1982) reported at least three kinds of proteases in fish muscle: acid, neutral, and alkaline proteases. Acid protease (cathepsin D) was probably the only fish muscle protease recognized before 1960 (Makinodan et al., 1982). Since then, endogenous muscle proteases exhibiting activity at pH 2-8 have been found in a wide variety of fishes (Makinodan et al., 1979, Ting et al., 1968; Makinodan and Ikeda, 1969).

Several researchers have suggested that the cathepsins in fish have little influence on the quality of intact fish tissue during cold storage. Geist and Crawford (1974) found that cathepsin activity in three species of sole did not substantially influence the organoleptic quality of fillets. Makinodan et al. (1982) reported that their results refute the possibility that acid proteases participate in autolysis of intact fish muscle. An alkaline protease active optimally at pH 8.0 and at 60-65<sup>o</sup>C was found to be widely distributed among fish (Makinodan and Ikeda, 1969). Studies have shown that an alkaline protease with similar properties was responsible for the deterioration of the textural strength of heat-processed fish gels (Lanier et al., 1981; Deng, 1981; Cheng et al., 1979; Su et al., 1981; Lin and Lanier, 1980). The integrity of actomyosin in comminuted fish tissue is critical to the formation of firm heat-set gels (Iwata et al., 1979; Suzuki, 1981). The activation of an alkaline protease in minced croaker gels heat-processed at  $60^{\circ}$ C (optimum temperature for protease activity) led to the proteolytic degradation of actomyosin and consequently a weakening of the gel integrity (Lanier et al., 1981).

Powerful proteases, exhibiting specific activities 10-20 times higher than that occuring in hake and flounder, have been characterized in squid muscle (Stanley and Hultin, 1984a). Acid and alkaline proteases have been identified in <u>Illex illecebrosus</u> exhibiting activity over a pH range of 2.6 to 7.4 with pH optima at 3.0, 5.8, and 6.6. Rodger et al. (1984) reported the presence of an alkaline protease in mantle and tentacle tissue of <u>Loligo forbesi</u> with a pH optimum of 7.6 and temperature optimum of  $60^{\circ}$ C. They implicated this enzyme in the proteolytic degradation of structural links in comminuted squid during incubation at  $2^{\circ}$ C. This was manifested by a progressive decrease in the viscosity of squid minces over time. The investigators noted that the proteolytic activity in the raw minces had no effect on the organoleptic quality of the minces in the cooked state.

Autolysis is largely responsible for the rapid spoilage and quality deterioration that occurs in post-mortem squid muscle (Gora et al., 1973; Saki and Matsumoto, 1981; Leblanc and Gill, 1982). Squid proteins autolyze rapidly during isolation procedures (Migita et al., 1958). The combination of the two unique characteristics of squid muscle, a fragile structure of its

myosin molecules and its high protease activity, mandates the use of protease inhibitors and EDTA during the isolation proteins to avoid decomposition of myosin (Tsuchiya et al., 1978).

The specific protease activity in <u>L. opalescens</u> reached only half that of <u>I. illecebrosus</u> under similar frozen storage conditions. Stanley and Hultin (1984a) reported an inverse relation between proteolytic activity in frozen mantles of three different species (<u>L. opalescens</u> < <u>L. peali</u> < <u>I. illecebrosus</u>) and quality as determined by sensory evaluation (<u>I. illecebrosus</u> < <u>L.</u> <u>peali</u> < <u>L. opalescens</u>). It was confirmed that the myosin and paramyosin protein components of mantle tissue underwent proteolytic degradation during frozen storage at temperatures as low as  $-20^{\circ}$ C. Actin was found to be the muscle protein most resistant to proteolysis (Saki and Matsumoto, 1981; Stanley and Hultin, 1984a).

## Storage Stability of Squid

Numerous investigators have shown that <u>Loligo</u> species of squid are more resistant to deterioration during iced and frozen storage than <u>Illex</u> species (Stanley and Hultin, 1984a and 1984b; Gora et al., 1973). The sensory quality of <u>Illex</u> decreased by 30 % over 5 days of iced storage while that of <u>Loligo</u> was not significantly affected (Stanley and Hultin, 1982). The higher level of proteolytic activity of <u>Illex</u> than of <u>Loligo</u> was held accountable for the greater cook loss measured for <u>Illex</u> (water lost during cooking: <u>Illex</u> = 36 %, <u>Loligo</u> = 14 %; protein lost to the cook water: <u>Illex</u> = 41 %, <u>Loligo</u> = 31 %). Several mechanisms for protein alterations and toughening in frozen fish have been elucidated; partial freeze dehydration, "salting out" of proteins caused by freeze concentration of inorganic salts, cross linking of proteins by the oxidation products of lipids and fatty acids; and formaldehyde induced protein cross linking (Sikorski et al., 1976; Matsumoto, 1979; Shenouda, 1980). Equimolar quantities of formaldehyde (FA) and DMA are produced from the enzymatic reduction of TMAO by TMAO-ase. It is postulated that FA is involved in various chemical reactions that lead to a crosslinking of the proteins, and consequently is responsible for a toughening in fresh and frozen tissue during storage.

The combination of large amounts of TMAO and TMAO-ase activity in squid mantle tissue (Stanley and Hultin, 1981 and 1984b) is a criterion for including squid in the group of marine animals that are potentially capable of producing sufficient FA to cause muscle toughening during frozen storage. Stanley and Hultin (1984b) compared <u>Illex illecebrosus</u> with <u>Loligo opalescens</u> and found greater amounts of DMA and higher rates of DMA formation in <u>Illex</u>. These species differences were not attributed to inequities in enzyme levels, but rather to the presence of unknown initiators and inhibitors in squid muscle. Certain compounds have been identified as activators of DMA production in homogenates of fish and squid (Stanley and Hultin, 1984b). Parkin and Hultin (1982) found that TMA and DMA inhibited the reaction. Stanley and Hultin (1984b) demonstrated the presence of an inhibitor of DMA formation in <u>Loligo</u> spp. and an initiator in <u>Illex</u> spp. The

addition of boiled <u>Illex</u> homogenate to a homogenate of <u>Loligo</u>, caused a 35-fold increase in DMA production. The addition of boiled <u>Loligo</u> homogenate reduced DMA production in a homogenate of <u>Illex</u> by 40%.

The tougher texture determined by sensory and instrumental measurements of Loligo over <u>Illex</u> observed by Stanley and Hultin (1982) appeared to be inconsistent with the relative decomposition of TMAO in these species. Stanley and Hultin (1984b), however, postulated that proteolytic activity could have superceded the toughening reaction of FA. The peptides and free amino acids produced by the action of proteases could combine with FA and prevent crosslinking. Consequently, it was believed that the higher level of protease activity in <u>Illex</u> (Stanley and Hultin, 1984a) was responsible for the tender texture observed for this species.

## **Restructured Seafood Products**

Surimi based analogs are the fastest growing form of restructured seafood products in the United States. The total quantity of surimi based products consumed in the U.S. in 1985 reached 88.4 million pounds with the projected total consumption for 1986 set at 119.5 million pounds (Parker, 1986).

Surimi consists primarily, of the actomyosin protein fraction of finfish flesh. Deboned, minced fish flesh is washed several times with water to leach away the water soluble proteins; low molecular weight, non-myosin proteins; lipids; pigments; and flavor compounds. The concentration of actomyosin is increased

with each wash. Gel elasticity and resiliency is improved by increasing the actomyosin concentration. Water-soluble proteins hinder gel setting by interfering with the actomyosin cross-linking process and cause a weakened gel (Okada, 1964; Shimizu and Nishioka, 1974). The washed flesh is then blended with cryoprotectants (commonly, phosphates, sorbitol, and sucrose) to preserve the functionality of the proteins during frozen storage.

Research, recently completed by John French, has shown that gel strength and water holding capacity of surimi gels are significantly correlated with the level of tropomyosin (Anonymous, 1986b). Excessive leaching of tropomyosin and troponin during washing results in low gel strength and water holding capacity. The amount of tropomyosin in surimi was directly related to water holding capacity where as the combined levels of tropomyosin, troponin, and myosin were related with gel strength. No significant correlation was found between gel strength and water holding capacity. French indicated that gel strength was a result of a composite interaction of at least actin, tropomyosin and troponin in addition to the the recognized role of myosin in forming protein gels.

Sano et al. (1986) demonstrated that the addition of paramyosin (isolated from squid muscle) to marine meat gels such as Alaska pollock surimi gels, significantly increased elasticity, and gel strength. Yang and Yang (1986) reported that the addition of squid tentacle proteins to Atlantic pollock surimi gels significantly increased fracturability, hardness, adhesiveness, springiness, gumminess, and chewiness, whereas, cohesiveness remained unchanged.
### Solubilization of Actomyosin:

Complete solubilization of the fibrous myofibrillar protein is necessary for gel formation. Sodium chloride is very effective at solublizing myofibrillar protein. The main component of salt-solubilized protein is actomyosin, the essential component for gel formation (Suzuki, 1981). Optimal gelling properties in surimi are obtained with the addition of 2.5-3.0 % NaCl (Lanier et al., 1985). The minimum amount of NaCl necessary to solubilize myofibrillar protein of fish muscle is a function of pH. Two percent NaCl is required at pH 7 and the required amount of NaCl increases with a decrease in pH (Suzuki, 1981).

Phosphates play a role in the solubilization of myofibrillar protein by influencing pH and ionic strength of the minced concentrated protein. Bendall (1954) reported that a combination of pyrophosphate and NaCl more than doubled the water-holding capacity of meat tissue over that of NaCl alone at equivalent ionic strength. This was attributed to the synergistic action of polyphosphates and NaCl acting together to increase the efficiency of protein solublization. Young et al. (1987) found that in the absence of NaCl, sodium tripolyphosphate (STPP) decreased waterholding capacity (WHC) in cooked chicken patties, but in the presence of NaCl, WHC increased as the level of STPP was increased. They observed that STPP was most effective in improving WHC in an environment containing less than 3% NaCl.

Polyphosphates are converted to the active pyrophosphate moiety by the phosphatase enzyme in meats. The hydrolytic activity of muscle ATPase converts part of the added

tripolyphosphate to pyrophosphate which has a specific swelling effect on meat in addition to its pH effect and ability to split actomyosin (Bendall, 1954; Sherman, 1961; Yasui et al., 1964). Pyrophosphate actively dissociates actomyosin to actin and myosin (Ellinger, 1972). Since fish muscle has relatively low phosphatase activity, the direct use of pyrophosphate promotes the most dramatic effect on texture and water binding ability of minced fish gels (Lanier et al., 1985). Polyphosphates may reduce the viscosity of surimi pastes excessively thus complicating gel formation. Addition of calcium chloride with polyphosphates will prevent excessive fluidity in surimi pastes and also increase the gel strength of the final product (Akahane, 1983).

### <u>Gel\_Transformations:</u>

Surimi is comminuted with NaCl to form protein sols. These protein sols can undergo several distinctly different gel transformations: low temperature setting, high temperature setting, and cook gelation (Lanier, 1986). Cook gelation involves heating the protein sol to >80°C (cooking temperature) for a short period of time to produce an opaque gel with firm and elastic texture. Suzuki (1981) proposed that thermal denaturation (unfolding) of the proteins exposes radical groups which can form intermolecular linkages (hydrogen, hydrophobic, and disulfide bonding) resulting in a network structure. Jiang et al. (1986) demonstrated the critical role that disulfide bonds play in the formation of minced fish gels. The low gel forming ability of frozen fish was attributed to the loss of reactive sulfhydryl

groups during frozen storage. Sulfhydral groups were recovered by adding reductants as the fish was ground. The pH of the minced fish was neutralized and oxidants were added during processing into the final product to induce the reformation of disulfide bonds which reinforced the protein network structure of the gel.

A different kind of sol-gel phenomenon has been identified as setting ("suwari", Japanese). Translucent gels are formed when protein sols are subjected to low temperature setting (holding near 0°C for 12-24 hours) and high temperature setting (near 40°C, 1 hour) (Wu et al., 1985a). Fish protein that has been set previous to cook gelation will possess stronger textural properties than if cooked directly from the raw state (Lanier et al., 1981). Both setting methods are used during the commercial manufacture of fish gel products to produce unique textural characteristics (Okada, 1981).

The mechanism for these two types of setting is dissimilar (Kim et al. 1985). Wu et al.(1985a) postulated that high temperature setting may consist of thermal denaturation (unfolding) of particular regions of myosin with subsequent formation of a network structure through aggregation of unfolded molecules. Results indicated that intermolecular hydrophobic interactions rather than disulfide bonding were responsible for gel formation at higher temperatures. Gel formation during low temperature setting did not appear to be related to thermal unfolding of proteins (Wu et al., 1985a). The results of Lanier (1986) indicated that disulfide bonding participates in gel formation during low temperature setting. The results also showed

a wide variation in the gel forming ability of muscle proteins among different fish species. Shimizu and Nishioka (1974) found a strong relation between heat stability of actomyosin of fish muscle and setting ability or suwari gel forming ability.

A degradative gel-transition phenomenon identified as "modori" by the Japanese, has been observed in surimi made from various fish species. It is characterized by a deterioration of gel structure as the fish gel passes through the 50-70°C range during heating and has been attributed to the action of alkaline proteases (Makinodan and Ikeda, 1969, 1977; Lanier et al., 1981). The origin of these proteases in most species of fish is the gut. The proteases are absorbed into the flesh during iced storage of whole fish or the flesh is contaminated during the deboning process with residual organ tissue adhering to the frames (Su et al., 1981a; Lanier, 1984). In other fish, proteases are endogenous to the muscle (Lanier, 1986).

# Texture and Ingredients

The texture of surimi gels can be modified by incorporating various ingredients: water, fats, starches, gums, and protein adjuncts, into the final formulation. Commercial surimi products presently on the market have 5-20 % starch added to increase the gel strength. Potato, wheat, sweet potato, and corn starches are most commonly used (Suzuki, 1981). The reinforcing effect of starch on surimi gels is attributed to the gelatinization of starch granules dispersed throughout the matrix. As the starch granules absorb water, they swell and fill the interstitial spaces

of the gel network. This "filler effect" impedes the free movement of water through the gel matrix, thus adding to its rigidity (Takagi and Shimidu, 1972; Okada and Yamazaki, 1959; Wu et al., 1985c). Wu et al. (1985c) demonstrated that potato starch is most effective in increasing the rigidity of surimi gels because the granules absorb more water and swell far greater than the granules of other starches. Kim and Lee (1985) found that high amylopectin starches (potato and waxy maize starch) produce cohesive gels in comparison to the brittle and weak gels formed by low amylopectin starches such as corn starch. Potato starch produced the firmest and most cohesive surimi gels of all the commercially available starches tested.

The fact that pregelatinized starches do not enhance the rigidity of surimi gels demonstrated the necessity for gelatinization of the starch to take place in the fish paste (Wu et al., 1985c). Okada and Yamazaki (1959) demonstrated that aldehyde treated starch was more effective at enhancing the strength of surimi gels than unmodified starch. Oxidized and acid modified starches were less effective gel reinforcers than unmodified starch. Gels prepared with unmodified starch have poor freeze-thaw stability (Lee, 1984). Lee (1986) reported that this was particularly true for corn and waxy maize starches which underwent severe retrogradation during frozen storage. Upon thawing the gels were high in expressible moisture and exhibited extensive syneresis which was accompanied by an increased rubbery texture.

The addition of modified starch (cross-linked type) improved the freeze-thaw stability of surimi gels but weakened the gel strength. To obtain a desirable balance between gel strength and freeze-thaw stability, equal proportions of unmodified and modified starches may be used in combination with egg white. The inclusion of oil (2.5 %) in the formulation also improved freeze-thaw stability. Due to their dissimilar structural compositions, molded products underwent more drastic freeze-thaw changes than fiberized products. Lee (1986) further explained that molded products take on a soggy and spongy texture because most of the drip from ice crystals was retained in the body whereas fiberized products only increased in rubberiness because the drip was not retained. Cooked products were more stable to freezing because thermal setting tightened the protein gel network which reduced damage to the matrix from ice crystal formation.

The addition of ingredients that bind up water or fat and impede its free movement through the fish gel matrix without interfering with the gel network formed by the muscle proteins is the key to ingredient-induced enhancement of gel strength (Lanier, 1986). The structure of fish gels may be reinforced to varying degrees by adding wheat protein or albumins such as egg white, whey protein concentrate, and soy protein concentrate. Egg white, soybean protein, and whey protein concentrate have been shown to increase the gel-strength of fish gels, however, their effects were eliminated when the water content was adjusted to that of the control which contained no additives (Burgarella et al., 1985; Iso et al., 1985). This indicated that protein adjuncts do not

contribute to the network structure of the fish gel. The increase in gel strength was attributed to a "filler effect" similar to that exhibited by starch. Bugarella et al. (1985) found that the gel-strengthening effects of the protein adjuncts were additive and there was no apparent synergistic interaction with the fish protein. Minced fish gels containing egg white were less rigid than those containing whey protein. Thermal scanning rigidity monitor tests revealed that egg white interfered with high temperature  $(40-50^{\circ}C)$  setting of fish proteins to a greater extent than whey proteins. Since setting greatly increased the cohesive properties of the gel, this interference resulted in reduced firmness of fish-egg white gels compared to fish-whey protein gels. In addition to contributing unique textural properties to the fish gel, egg white also improved whiteness and glossiness.

Lee (1986) explained how a desirable texture was obtained in a surimi product with the proper starch-egg white combination. Addition of up to 10 % starch to surimi gels increased cohesiveness and rigidity while it slightly decreased elasticity and firmness (respectively, in terms of; failure force, penetration force, percent elastic recovery, and compressive force at 50 % deformation without rupture). In contrast, fish-egg white gels were significantly less elastic, firm, and cohesive than fish-starch gels. Partially heat-set gels containing egg white were more elastic and pliable (conducive to forming and molding), however, they became brittle and less elastic after the final cooking. Starch increased rubberiness whereas egg white reduced rubberiness. When the two were used together in surimi products

each influenced the texture of the final product in accordance to its own unique properties. Since starch and eqq white did not have a synergistic effect on the texture of surimi gel, the texture of the final product was manipulated by adjusting the ratio of starch and egg white so that a meaty texture was achieved. The starch and egg white composition of three typical seafood analogues fabricated by Japanese manufacturers are; 5 % and 8 % for crab, 4.3 % and 5 % for scallop, and 11 % and 4 % for shrimp analogues respectively (Lee, 1986).

#### Moisture and pH

Moisture content greatly influences the texture of surimi gels. Factory ship top-grade surimi contained 82 % moisture before the addition of cryoprotectants. After 8-9 % cryoprotectants were added, the moisture level was 75-79 % (Lee. 1986). The textural effects of moisture were dependent upon its interaction with other ingredients in the surimi product formulation. The moisture content in commercial formulations ranged from 72-78 % depending upon the nature of the final product. Generally, the higher the moisture level, the more susceptible a product was to freeze-thaw destabilization (Lee, 1986). Lee and Toledo (1979) reported that the strength of cooked gels made from comminuted Spanish mackerel (unwashed) decreased gradually as the moisture level was increased to 79 % and dropped drastically with moisture levels in excess of 79 %. The results suggested that products are less cohesive below a critical moisture content.

In the commercial production of surimi in Japan, the pH of the flesh was maintained between 6.5 and 7.0 throughout the process in order to retain the water holding capacity of the proteins. The gel strengths of surimi based products were influenced by pH. The strongest gels were obtained when the pH was maintained between 6.5 and 7.5 (Okada, 1985). The optimum pH range for solubilization of actomyosin was found to be 6.5-7.0 (Knipe et al., 1985). The pH of surimi-based products is commonly adjusted with phosphates and/or sodium carbonate. Pyrophosphate was the more effective than hexametaphosphate, tripolyphosphate, metaphosphate, and acid pyrophosphate in raising the pH of comminuted meat (Shultz et al., 1972). The ability of pyrophosphates to increase the water-holding capacity of cooked meat was greatest when the pH was above 6.5 (Hellendoorn, 1962).

### <u>Flavorings</u>

Akahane (1983) described the interaction of flavor additives used in Japanese surimi products. Natural flavors are usually not strong enough and must be strengthened with artificial flavors. Extracts are condensed from natural materials and contain many of the naturally occuring flavor potentiators. A finished flavor for any product must be blended from several natural flavors, artificial flavors, extracts and potentiators. Japanese seafood analogues made from surimi normally contain 0.3 to 0.5 % flavor additives. Akahane (1983) recommended levels of 0.5 to 1.2 % to suit American tastes.

Soluble nitrogen compounds such as amines, amino acids and nucleotides are the best flavor potentiators for seafood flavors. Monosodium glutamate is a popularly used flavor potentiator. Glycine, which occurs naturally at substantial levels in shellfish and crustacea meats, is an important flavor additive in scallop, shrimp, and crab analogues. Hydrolyzed vegetable protein and hydrolyzed animal protein are mixtures of amino acids and function as flavor potentiators. Nucleotides such are sodium inosinate and sodium guanylate are very effective flavor potentiators, however, precautions must be taken to prevent the enzymatic conversion of inosine monophosphate to the inactive inosine form by the phosphatases present in surimi. Small amounts (0.03 %) of sodium succinate are sometimes used to impart a fish taste. Milin may be added to mask fishy odors and add a glossy sheen. Spices may be added for color. flavor, and to mask flavors and odors. Onion. laurel, sage and ginger are effective at masking the trimethylamine taste and odor of fish.

### **Restructuring Techniques:**

Akahane (1983) outlined the innovative restructuring techniques that are used by Japanese food companies to form seafood analogues from surimi. Surimi is blended with salt to form a paste and ingredients such as water, starch, egg white, and flavoring are mixed in (Akahane, 1983). The process for fabricating crab leg analogues involves spreading the paste into a sheet, 1.2 to 1.5 mm thick, on a conveyer belt and transforming it to a gel by high temperature setting ( $40^{\circ}C$  for 20 minutes). The

gel sheet is cut into thin noodle-like strings with bar knives. The cutting depth is adjusted to 1 mm for a 1.2 mm thick gel sheet. The cut gel sheet is rolled into a cylinder resembling the long fibrous structure of rope and then wraped with a thin (1/2 mm thick) gel sheet of surimi that was previously formed on plastic film. The outside gel sheet used for wrapping is softer (contained more water) than the fibrous surimi gels composing the inside and is often colored red. After vacuum packaging the crab legs are cooked at 90°C for 30 minutes to firmly fix the gel.

Scallop analogues are made with the same process used for crab legs except the cut gel sheets are rolled into larger diameter cylinders and sectioned into shorter lengths. Shrimp analogues are fabricated in two different ways. The quick and easy method is to pour surimi paste into a shrimp mold and cook it, forming a homogenous gel. The other method produces a fibrous structure that more accurately resembles a true shrimp-like texture. Fibers are made by cutting gel sheets of surimi into thin strings and mixing these with raw surimi paste. The mixture, composed of 70 % fibers and 30 % paste, is formed into shrimp molds and cooked (Akahane, 1983). A meat-like texture can be simulated in surimi products through slow freezing (-13<sup>o</sup>C in 24 hours) (Kammuri and Fujita, 1985). This promotes the growth of large ice crystals in the surimi gel, which produce a fractured texture similar to that of meat.

#### MATERIALS AND METHODS

### <u>Raw Materials</u>

Whole food-grade squid, <u>Loligo opalesence</u>, (source: California waters; count: 22/kg) were procured from a local seafood processor and wholesaler in Warrenton, Oregon. The squid had been plate frozen in five pound boxes soon after capture and stored at  $-18^{\circ}$ C for approximately 6 months before processing.

### Squid Processing

### Cleaning and Dewatering

Approximately 10 kg of frozen, whole squid were thawed at  $4^{0}$ C for 24 hours. Mantles were eviscerated and skinned by hand. The tentacles were not skinned, only the beaks were removed. Whole squid, cleaned mantles, and cleaned tentacles were drained in a colander for 15 minutes and weighed for yield determinations. Cleaned mantles and tentacles were coarsely chopped in a food processor.

The chopped squid flesh was dewatered by wrapping one kg lots in cheesecloth and pressing between perforated aluminum trays with an industrial 40 ton hydraulic press. Pressure was applied for about five minutes until liquid stopped dripping from the squid and the moisture content of the squid was reduced to approximately 78%. Yields for cleaned mantles, cleaned tentacles, and dewatered minced squid were recorded.

### **Experimental Product Preparation**

Products were prepared to evaluate the adjustment of product pH and to establish the effectiveness of protein adjunct. Product preparation was repeated twice using a separate batch of whole squid (5 and 10 kg, respectively) for each investigation.

Adjustment of pH Two five 1b blocks (ca. 5 kg total) of squid were thawed, cleaned, chopped, and dewatered as described previously. Four lots (500 gm each) of dewatered, chopped squid flesh (70 % mantle and 30 % tentacle flesh by wt.) were minced in a food processor for 30 seconds. Phosphates [mixture of Na acid pyrophosphate, Na pyrophosphate and Na polyphosphate; Brifisol 414, BK-Ladenburg Corp., North Hollywood, CA (0.5 %)], NaCl (0.5 %), and varying levels (0.0 %, 0.25 %, 0.50 %, and 0.75 %) of Na<sub>2</sub>CO<sub>3</sub> (anhydrous powder; Mallinckrodt Chemical Works, St. Louis) were minced into each lot of squid for an additional 30 seconds. Mincing was stopped after 10 second periods to scrape down the sides of the processor container. The level of NaCl was kept at a minimum (0.5%) because excess Na in the diet is believed to contribute to high blood pressure in some people (Marsh et al., 1980).

Plastic food trays (12 cm x 9.5 cm and 2.5 cm deep) were filled with a recorded weight of squid paste (ca.  $350\pm25$  g). Care was taken to exclude air pockets. The filled trays were vacuum sealed (8" Hg) in moisture-vapor proof film. After a 4 hour setting period at  $4^{\circ}$ C, the formed pastes were immersed in a  $90^{\circ}$ C water bath for 50 minutes and then cooled in an ice water bath for 15 minutes. The gels were removed from the vacuum package,

blotted dry with paper towels and weighed for yield determinations.

Protein Adjunct Four five lb blocks (ca. 10 kg total) of squid were thawed, cleaned, chopped, and dewatered as described previously. Four separate lots (500 g each) of squid were minced with 0.5 %; NaCl, condensed phosphate, and  $Na_2CO_3$  as described previously for the pH adjustment investigation. A different protein adjunct, sodium caseinate (EM HV, De Melkindustrie Veghel BV., Veghel, Holland) or egg white (Milton G. Waldbaum Co., Wakefield, Nebraska) or soy protein isolate (Ardex SP-W, Archer Daniels Midland Co., Decatur, Illinois) was added at a level of 2 % by weight to each lot of squid paste and homogenized in a food processor for one minute. The 2 % adjunct level was chosen because preliminary sensory tests showed that SPI at levels >2 %, imparted a strong soy flavor to the gels. Homogenizing was stopping after each 15 seconds period to scrape the sides of the food processor container. One lot, the control, contained no protein adjunct. The control preparation and formulations containing the different protein adjuncts were subjected to two setting treatments. Squid pastes were held at  $4^{\circ}$ C for 4 and 24 hours before they were formed and heat-set.

# **Restructuring Squid Product**

8

Protein adjunct paste formulations were prepared as described for the protein adjunct investigation and 5 % by weight of dried potato starch (Horowitz Bros. & Margareten, Long Island City, New York) was added to each and blended in a food processor for one minute using procedures outlined previously. This starch-protein gel sol was restructured to form the final product.

Lampila et al. (1985) fabricated turkey loaves with desirable, fibrous and cohesive, meat-like textures from mechanically deboned turkey meat. A portion of the minced turkey was formed into heat-gelled fibers and incorporated into a carrier of ungelled mince.

This restructuring technique, with minor modifications in fiber diameter and heat-setting method was applied to squid in this investigation. The diameter of the fibers was reduced to make them easier to form into small patties. Fibers were heat-set with steam so that the moist heat would prevent dessication and surface cracking.

Fibers were prepared by extruding the gel sol through a custom-made dye fitted onto a sausage stuffer. The long spaghetti-like strands (2 mm diameter) were extruded directly onto trays and steam cooked at 100°C for 10 minutes, cooled at room temperature (20°C), and chopped into small pieces (approximately 1 mm length) in a food processor.

Gel sol (2 parts) was mixed into the fibrous chunks (3 parts) forming a matrix of carrier material and fibers. This proportion of gel sol provided sufficient cohesiveness to bind the fibers together and prevented the matrix from sticking to the forming machine. The matrix was formed into fish-shaped patties (14 g each) using a Hollimatic No. 200 food forming machine. The patties were wrapped in wax paper, sealed in plastic bags and

stored at  $-18^{\circ}$ C for three weeks until sensory evaluations were carried out.

# Evaluation Procedures

### Cooking and Cool Loss

Heat set gels were removed from the vacuum sealed plastic trays after cooling and blotted dry with paper towels. The gels were weighed and cook-cool loss was expressed as the percent weight difference before and after heating.

# Moisture Determination

The moisture content of the squid pastes and gels was determined by drying preweighed samples  $(8\pm1 \text{ gm})$  of paste or minced gel in a  $100^{\circ}$ C oven for 24 hours. Moisture was expressed as percent weight difference of the samples before and after drying.

### Determination of pH

The pH of each squid formulation was measured before and after heating. Five grams of paste or gel were homogenized with 45 g distilled water in an Osterizer blender for one minute and the pH of the homogenate was measured with an Orion Research Digital pH Meter, Model 701 fitted with a Corning combination pH electrode.

#### Proximate Composition

The proximate composition (moisture, protein, lipids and ash) of the two lots of raw squid (mantles and tentacles) and samples of restructured squid product containing EW were determined in triplicate by the standard AOAC (1984) methods.

## Instron Measurements

Compression Measurements The compression measurements, as described by Pan et al. (1981), were carried out with an Instron Universal Testing Instrument (Model TM-M). Test samples were cut from gels using a cork bore and razor blade to a standard size of  $2 \times 2.5$  cm (length x diameter) and a weight of 8-10 g. Gel samples were enclosed in containers, to minimize loss of moisture. and held at room temperature  $(22^{\circ}C)$  for 3 hours before the compression test was conducted. Each gel sample was placed on a stationary platform and compressed longitudinally by an overhead compression anvil (5 cm diameter) that was attached to a 50 lb compression load cell which hung from the moving cross bar (X-head speed, 5 cm/min). The samples were compressed 1.8 cm (90 % of their original height) and the plunger was immediately withdrawn. The force-time curves were plotted on a chart recorder (chart speed, 50 cm/min). A brief downward deflection in the rising slope of the force-time curve, depicted a break in the gel or gel failure.

Break force (expressed in g) of the gel was measured at the point of gel failure on the curve. Deformation (expressed in mm), the distance that a sample is compressed when failure occurs, was

measured by the horizontal distance along the baseline of the curve, from the start of compression to the point of failure.

Expressible Moisture Measurements were carried out on gels containing protein adjunct simultaneously with the compression measurements according to procedures described by Lee and Kim (1985) and Regenstein (1984). A piece of pre-weighed, Whatman, No. 1 filter paper (10 cm<sup>2</sup>) was placed under each gel sample prior to compression. The moisture expressed from the sample during 90 % compression was absorbed by the filter paper and measured by the difference in weight of the filter paper before and after compression. The expressible moisture was reported as the percentage of moisture in the sample that is expressed after 90 % compression, (wt. of moisture expressed from sample/wt. moisture in sample x 100).

# Sensory Evaluation

Sensory evaluations were conducted at the Sensory Evaluation Laboratory of the Department of Food Science and Technology at Oregon State University under the supervision of Dr. Mina McDaniel. Three types of squid patties, each containing a different protein adjunct, were evaluated by a trained and a consumer preference panel.

<u>Trained Descriptive Panel</u> An eight member descriptive attribute panel was trained to identify firmness, cohesiveness, juiciness, and chewiness as defined in Table 3. During two training sessions, the panelists compared the textural attributes of various food items with those of the squid patties and selected

the most appropriate food item to serve as a reference for each attribute. Patties were segregated by protein adjuncts and deep fried (185<sup>0</sup>C, 75 seconds) six at a time in separate batches of oil to prevent any possible flavor carry over. After cooling to room temperature (20<sup>0</sup>C), the patties were cut into bite-size pieces  $(1.5 \text{ cm}^2 \text{ by 1 cm thick})$ . Samples were evaluated by magnitude estimation as described by Moskowitz (1983). Panelists compared the magnitude of each sensory attribute in the three types of patties with that of the appropriate reference sample. The magnitude of each sensory attribute was estimated by assigning a number proportional to the standard magnitude rating of the attribute in the reference sample. For example, the standard rating of the reference sample for firmness was 50. The panelist compared the firmness of the squid patty with that of the reference and assigned the patty a magnitude estimation score for firmness; a 50 if it was equally firm, 100 if twice as firm, 25 if half as firm, or any other proportional number.

		l
Textural  attribute	Sensory Definition <sup>a</sup>	Reference sample
Firmness	Force required to compress between	Pork Sausage <sup>C</sup>
  Cohesiveness 	Degree of compression by the molar teeth before the sample breaks apart	Pork Sausage <sup>C</sup>
Juiciness	Amount of moisture released from sample	Hot dog d
Chewiness	Number of chews necessary to	None
'		

Table 3. Sensory attributes and selection of reference samples

a From Szcezesniak et al. (1963) Sample size - 1 cm thick by 1.5 cm square

d"Oscar Mayer Hot Dogs", unheated, served at room temperature (20<sup>0</sup>C)

<sup>&</sup>lt;sup>C</sup>"Swift Premium Brown'N Serve Pork Sausage Patties", deep fried (180<sup>0</sup>C, 3 minutes), cooled to room temperature (20°C) and cut into bite-size pieces

<u>Consumer Preference Panel</u> The consumer panel consisted of 34 people that were solicited from the staff of Department of Food Science and Technology. The panelists, having no previous coaching, evaluated the patties on a 9-point hedonic scale (scale range: 9, extremely acceptable; 5, borderline acceptability; 1, extremely unacceptable) for overall appearance, texture, flavor, desirability, and intensity of seafood flavor. Squid patties were deep fried as described previously and kept under heat lamps for not more than ten minutes prior to serving. Each panelist evaluated three whole patties, each patty containing a different protein adjunct.

# Statistical Analysis

Analysis of variance was conducted with the Stats Plus program (Human Systems Dynamics) on an Apple IIe computer. Analysis of variance using one-way and factorial designs, was used for appropriate data. When analysis of variance revealed a significant effect ( $P \ge .05$ ), the least significant difference (LSD) test was employed to determine differences between level and treatment means.

Analysis of the trained sensory panel magnitude estimation scores was carried out in the manner described by Moskowitz (1983). The variability of the magnitude estimation scores was reduced by dividing each score in a set by the geometric mean of the set. A set consisted of the estimation scores from a single panelist for the magnitude of a certain attribute in all three patty samples. The geometric mean was the cubed root of the

product of the three scores. When analysis of variance of these modified scores revealed a significant level effect ( $P \ge .05$ ), the LSD test was employed to determine significant differences between treatment means.

#### **RESULTS AND DISCUSSION**

#### Adjustment of Gel pH

# Effect of Sodium Carbonate on Gel pH

Incremental additions of sodium carbonate to comminuted squid produced a linear (r = .99;  $P \ge .001$ ) increase and significant differences ( $P \ge .001$ ) in the pH of raw and cooked gels (Table 4). Each 0.25 % increment of sodium carbonate, up to a level of 0.5 %, produced an increase in the raw gel pH of about 0.6 units. Increasing the level of sodium carbonate from 0.5 % to 0.75 % affected an increase in pH of only about 0.2 units which was not significant (P=.05). Cooking increased the pH of squid gels from .23 to .59 units. Gels with higher raw pH levels (7.49 and 7.72) underwent a greater rise through cooking than gels with a lower pH (6.21 and 6.88).

An increase in the pH of meat as a result of heating was observed by Wierbicki et al. (1957). Hamm and Deatherage (1959) attributed the rise in the pH of meat that resulted from heating to a loss of acidic functions (carboxyl groups) on the meat proteins. They found that the effect of heating on pH was more pronounced in meat with a lower raw pH where the presence of acidic functions was more influential. The reversed relationship between raw pH and change in pH due to cooking observed in this investigation for squid gels may have been a function of the complexing influence of Na+ ion that accompanied sodium carbonate additions.

### Effect of Gel pH on Cook-Cool Loss and Moisture Content

The water holding capacity of cooked squid gels increased as the pH of the gels rose in response to incremental additions of sodium carbonate (Table 4). Cooked gel moisture content increased  $(P \ge .007)$  and cook-cool loss decreased  $(P \ge .001)$  in a significant manner with respect to the level of sodium carbonate added. The moisture content of gels containing 0.00 and 0.25 % and those containing 0.50 and 0.75 % sodium carbonate were equal (P = .05). The cook-cool loss from gels containing 0.00, 0.25 and 0.50 % sodium carbonate differed, while those containing 0.50 and 0.75 %

These results were in agreement with those of Bouton et al. (1971) who showed that the cook-cool loss of comminuted mutton at  $90^{\circ}C$  decreased linearly with increasing pH, while the water holding capacity improved as the pH was elevated from 6.0 to 7.0. Hamm and Deatherage (1960) confirmed that the water holding capacity of meat increased as the pH was raised above the isoelectric point (I.P. = 5.0 for meat). It was asserted that the electrostatic attraction between the protein molecules was eliminated as the alkalinity of the meat increased. The protein net charge was increased, which resulted in an increased repulsion of the peptide chains and consequently, an enlargement of the space between the protein molecules allowing greater penetration of water into the matrix.

Effect of Gel pH on Break Force and Deformation at Break

The pH increase produced by incremental additions of sodium carbonate significantly reduced the force ( $P \ge .012$ ) required to break and the deformation distance ( $P \ge .01$ ) at break of squid gels (Table 4). While pH significantly altered break force, the

Table 4. Effect of varying levels of sodium carbonate on the moisture content, cook-cool loss, break force and deformation at break of squid gels.

		5	Sodium Carl	bonate (%)	l
Parameter		0.00	0.25	0.50	0.75
  pH (pre-cook)	  Mean   S.D.	6.21 <sup>a</sup> .03	6.88 <sup>b</sup> .12	7.49 <sup>cd</sup> .03	7.72 <sup>d</sup>
pH (post-cook)	Mean S.D.	6.44 <sup>a</sup> .03	7.07 <sup>b</sup> .09	7.96 <sup>C</sup> .03	8.31 <sup>C</sup> .06
   Moisture (%)	Mean S.D.	71.95 <sup>a</sup> .55	73.10 <sup>a</sup> .30	74.90 <sup>b</sup>	75.50 <sup>b</sup> .20
Cook-Cool Loss (%)	  Mean  S.D.	27.2 <sup>a</sup> .1	20.5 <sup>b</sup>	   17.2 <sup>C</sup>   .2	15.8 <sup>C</sup>
Break Force (g)	  Mean  S.D.	1907 <sup>a</sup> 157	1629 <sup>a</sup> 84	1153 <sup>b</sup> 112	898 <sup>b</sup> 77
Deformation (mm)	Mean	12.4 <sup>a</sup> .4	10.3 <sup>b</sup>	9.9 <sup>b</sup>	10.0 <sup>b</sup>
Parameter		F-value	Significance		
  pH (pre-cook)  pH (post-cook)  Moisture (%)  Cook-Cool Loss (%)  Break Force (g)  Deformation (mm)		87.04 245.88 24.1 150.47 16.54 18.53	$P \ge .001 \\ P \ge .001 \\ P \ge .007 \\ P \ge .001 \\ P \ge .012 \\ P \ge .010$		

n=2

Mean values in row with same exponent letter did not vary (P=.05)

force required to break gels containing 0.00 and 0.25 % and those containing 0.50 and 0.75 % sodium carbonate were equal (P=.05). The greatest deformation at break was observed for gels possessing a pH associated with no addition of sodium carbonate (P=.05). Higher pH levels produced by additions of sodium carbonate yielded lower deformation distances that did not vary (P=.05).

Raising the pH of the squid gels with sodium carbonate enhanced the ability of salt and polyphosphates to solubilize the proteins (Knipe et al., 1985) leading to an increased hydration of the polypeptide chains. This allowed more moisture to be retained within the protein network structure of the gel that was formed upon cooking. In the absence of additional gel forming and stabilizing ingredients, the loss of gel strength was a direct consequence of increased moisture in the gels (Lee and Toledo, 1979).

The adjustment of squid gel pH by the addition of sodium carbonate produced a significant and inverse relationship between the moisture content and both break force and deformation distance  $[r= 0.99 \ (P \ge .001)$  and  $0.90 \ (P \ge .005)$ , respectively]. Pan et al. (1981) investigated the effects of ingredients on the texture of minced squid products and found an inverse linear relationship between gel strength and moisture content. These researchers reported that as pH was adjusted from 6.0 to 6.7 with polyphosphates, break force and water retention (by centrifugation method) increased. Above pH 6.7 a decrease in break force and an increase in water retention occurred. Hellendoorn (1962) reported that the ability of pyrophosphates to increase the water-holding

capacity of cooked meat is greatest when the pH is above 6.5. The ionic strength of squid gels, as well as pH, was altered by additions of sodium carbonate. Both of these two physical characteristics have been shown to influence the textural properties and water holding capacity of comminuted fish (Weinberg et al. 1984, and Regenstein, 1984).

# Protein Adjunct

# Effect of Holding Time

Holding squid gel sols at  $4^{\circ}$ C for 4 and 24 hours before forming and heat-setting into gels had a significant effect on the break force (Table 5) and expressible moisture (Table 6) of squid gels containing different protein adjuncts. The force required to break the gel was decreased (P=.001) by extending the holding time from 4 to 24 hours. Inspection of individual treatment means revealed a significant (P=.05) reduction in break force with the extension of holding time for gels either containing soy protein isolate adjunct or the control. Break force was reduced for the gel containing egg white, but not to a significant extent. Extending the holding time increased (P=.006) expressible moisture. No individual adjunct gels varied (P=.05) in their amount of expressible moisture with respect to holding time. Holding time did not affect the moisture content of heat-set gels (Table 7), the cook-cool loss (Table 8) or the deformation distance (Table 9).

The lower break force and elevated quantities of expressible moisture observed for an extended holding time could have been produced by autolytic proteolysis of squid myosin. Stanley and Hultin (1984a) identified powerful proteases in squid muscle. Rodger et al. (1984) reported that an alkaline protease (pH optimum, 7.6) in mantle and tentacle tissue of squid was responsible for the proteolytic degradation of structural links in raw comminuted squid at  $20^{\circ}$ C. Autolysis of the raw comminuted squid was not found to affect the perceived texture of the cooked system or its thermal transitions as measured by differential scanning calorimetry. A combination of high protease activity and fragile myosin structure predisposes squid to rapid autolysis of the structural proteins (Tsuchiya, 1978). Stanley and Hultin (1984a) showed that myosin and paramyosin protein components of squid mantle tissue undergo proteolytic degradation during frozen storage at  $-20^{\circ}$ C. Instron shear-force measurements, revealed that proteolytic enzymes do indeed have the ability to influence the textural quality of raw intact squid muscle, in vitro, at  $5^{\circ}$ C.

The action of proteases on squid gel sol proteins is supported by the fact that break force values did not vary significantly with respect to holding time for squid gels containing egg white. Protease inhibitors, ovoinhibitor and ovomucoid, present in egg white (Richardson, 1976) may have played an important role in preventing the degradative action of the alkaline proteases on the myosin protein components of the squid gel sol. Consequently, the gel-forming ability of the squid gel sols may have been maintained over the 24 hour holding period. Groninger et al. (1985) reported that addition of dried egg white

in conjunction with potassium bromate yielded optimum gel strength and reduced protease activity by 95 % in Pacific hake surimi.

Kawana (1983), working with washed and minced shortbelly rockfish flesh, found that a fish gel sols containing 5 % egg white produced a more firm and elastic gel on cooking at 85<sup>0</sup>C than gel sols without egg white. It was surmised that protease inhibitors present in egg white prevented the muscle degrading action of proteolytic enzymes that are inherent to short-belly rockfish muscle.

Su et al. (1981) reported that the alkaline protease activity in various tissues of croaker (including muscle tissue) was destroyed at temperatures >70<sup>0</sup>C. Higher levels of protease activity in raw comminuted croaker muscle were associated with reduced hardness values for fish gels that were cooked at  $60^{\circ}$ C. Lanier et al. (1981), working with minced fish, found that autolysis during cooking did affect the gel strength. When potato protease inhibitor was added, proteolytic activity during cooking decreased and gel strength of the cooked gels significantly increased. While ovomucoid was reported to be an effective protease inhibitor in minced croaker (Lin and Lanier, 1980) there was no indication that addition of ovomucoid to minced croaker significantly increased the strength of the heat-set gels (Lanier et al., 1981). These studies were designed to examine the protease activity during cooking. The present study dealt with possible proteolysis occurring in the raw sol during an extended period of time at 4<sup>°</sup>C. The squid formulations were heated rapidly to 90<sup>°</sup>C by submersion into a hot water bath, thus avoiding proteolysis during lower cooking temperatures (<70°C).

While the action of proteases offers a reasonable explanation for reduced gel strength, the contribution of egg white protein to the protein network structure of squid gel offers an additional explanation. Disulfide bonds play an important role in the protein network structure of minced fish gels (Jiang et al., 1986; Niwa, 1985). The addition of egg white constituted a significant contribution of sulfhydryl groups to the squid protein system. Upon heating, these sulfhydryl groups can form disulfide bonds which reinforce the gel structure. The superior gel reinforcing ability observed for the egg white adjunct may have reflected its contribution of reactive sulfhydryl groups to the squid sol.

A two-step heating method was found to be superior to the one-step heating method in producing firmer gels with higher moisture content for Alaskan pollock surimi (Niwa, 1985) and for gel products made from fractionated squid proteins (Sano et al., 1986). The two steps involve holding uncooked material at relatively low temperature ( $4^{\circ}$ C) before heating. The weak gel that forms at this low temperature is transformed into a very firm gel upon heating. If the structure of a low-temperature set gel is disrupted before heating, however, the resulting gel will be less firm than a gel of the same material that was formed by a one-step heating method (Lanier et al., 1982).

In this investigation, when squid sols were held at 4<sup>o</sup>C for 24 hours, cold temperature setting may have taken place resulting in the formation of a weak gel. This gel was disrupted, however, when the gel sol was transferred to plastic trays. Consequently the disrupted two-step gels were less firm than the one-step gels.

The contribution of additional sulfhyryl groups from egg white to the squid protein system could have enhanced the strength of the heat-set gel sufficiently to preclude the effect of disruption of cold temperature set gels on the final gel strength. The loss in gel strength that occurs when low temperature set gels are disrupted before they are heat set may not be of consequence when egg white is present in the system.

## Effect of Protein Adjuncts

Squid gels containing sodium caseinate did not exhibit a discernible breaking point on the force-time curves. Instead of fracturing when compressed, the squid gels containing sodium caseinate were completely flattened in one smooth motion. In contrast, the other squid gel samples showed a distinct breaking point on the force-time curves. These gel samples possessed some degree of fracturability. They maintained their integrity as the compresive force was increased to the point where it exceeded the cohesive forces of the gels and gel failure occurred.

Protein adjunct significantly ( $P \ge .026$ ) altered the force required to break gels (Table 5). The break force of gels were ranked control > soy protein isolate > egg white. The control gels possess superior (P=05) strength to squid gels containing either soy protein isolate or egg white. The strength of squid gels containing soy protein isolate and egg white were equal (P=.05). Inspection of individual treatment means revealed that squid gels containing soy protein isolate and egg white possessed significantly (P=.05) reduced break force values when subjected to a 24 hour over a 4 hour holding time. Break force values for squid gels containing all protein adjuncts were equal (P=.05) when subjected to a 24 hour holding time. The protein adjucts and holding time factors interacted in a significant (P $\geq$ .022) manner to alter break force values.

Protein adjuncts to squid gels significantly (p>.001) affected cook-cool losses (Table 8). Gels containing sodium caseinate lost the most weight during cooking and cooling (19.75 %) and was followed by the control (16.65 %) and squid gels containing soy protein isolate (12.41 %) and egg white (5.29 %). Cook-cool losses by the control and the squid gel containing sodium caseinate were equal (P=.05), but significantly (P=.05)greater than both gels containing soy protein isolate and egg white. The cook-cool loss of the squid gel containing egg white was less (P=.05) than that for the soy protein isolate. The differences between individual treatment means, with respect to protein adjunct, for the 4 and 24 hour holding times were identical. The factors of holding time and protein adjunct did not interact significantly with respect to cook-cool loss was observed.

The significant difference ( $P \ge .018$ ) observed in the moisture content of heat-set gels was largely a function of the lower moisture content of gels containing sodium caseinate (Table 6). The moisture content of the squid gels containing sodium caseinate was less (P=.05) than the control and the gels containing soy protein isolate. Control gels and those containing egg white and soy protein isolate contained equal (P=.05) amounts of moisture. The results indicate that sodium caseinate functioned poorly as a water binder in squid gels. Perhaps the actomyosin proteins of squid were salted out by the excessive Na<sup>+</sup> contributed to the protein system by sodium caseinate reducing their water holding capacity.

Protein adjuncts had no ( $P \le .05$ ) apparent effect on expressible moisture (Table 7). Although the procedure was sensitive enough to detect differences related to holding time, the sample size was probably too large and 90 % compression was insufficient to detect differences among adjuncts. The method to measure expressible moisture that was first developed by Grau et al. (1953) has been found to be satisfactory by subsequent research. This method entails sandwiching a gel sample (1 cm thick) between filter paper and pressing for a fixed amount of time with a prescribed constant pressure. The moisture expressed from the sample is then indirectly measured by the wet area on the filter paper. Suzuki (1981) reported that Iwata used this press method and observed an inverse correlation between sensory scores for gel strength of kamaboko and expressible moisture measured by the press method.

Squid gels containing sodium caseinate did not break when compressed precluding the measurement of deformation (Table 8). The remaining gels, with respect to protein adjunct, did not vary significantly ( $P \le .05$ ).

Several researchers have reported that protein adjuncts as well as starch function as fillers in fish gels. Burgarella et al. (1985) studied thermal transitions and rigidity of gels made from Atlantic croaker surimi containing up to 60 % dried egg white or dried whey protein concentrate. Fish gels containing whey protein concentrate had higher Instron rigidity values than egg white-fish gels. The researchers concluded from the results that egg white and whey protein concentrate enhanced the rigidity of surimi gels through a "filler effect". This was the same mechanism described by Suzuki (1981) and Wu et al. (1985c) for the enhancement of kamaboko gel strength by starch. The globular proteins (egg white and whey protein concentrate) absorbed water and filled the interstitial spaces of the fish protein gel matrix. The turgidity of the matrix was increased as a direct result of increased solids content and decreased moisture content. Iso et al. (1985) also attributed this same filler mechanism to the increased gel strength and elasticity of surimi gels containing egg white and soybean protein. It was demonstrated that the effects of the protein adjuncts were eliminated when the water content was adjusted to that of the control. This indicated that the additives do not contribute to the network structure of kamaboko but instead perform as fillers.

The results of this investigation suggest that egg white may play a more active role in enhancing the gel strength than simply acting as a filler. Squid gels containing egg white were subject to only small cook-cool losses and the break force values were relatively high in spite of a relatively high moisture content (74 %). Egg white may contribute disulfide linkages to the network structure of the squid gels. This direct involvement with the matrix of the gel may be the key factor that enables egg white to significantly enhance gel integrity.

ho.	lding times a	t 4°C.	1	1
Holding   Time   (hrs)	Protein   Adjunct <sup>1</sup>		     Mean <sup>2</sup>	
   <u>4</u>   	  Egg White (EW)  Soy Isolate (SPI)  Control (Cont)  Sodium Caseinate (Cas)		737 <sup>ab</sup>   888 <sup>b</sup>   1144 <sup>C</sup>   NB	32  30  72
   24 	  Egg White  Soy Isolate  Control  Sodium Caseinate		-    637 <sup>a</sup>   620 <sup>a</sup>   626 <sup>a</sup>   NB	83   63   3
	Analysis of	Variance: Fa	ctorial Design	
	 F-v	alue	Ranking of L	evel Means
  Adjunct (A) 	7.14 (Sig.	P <u>&gt;</u> .026)	Adjunct:  Cont <sup>a</sup> > SPI <sup>b</sup> > E	ь "Ъ
Time (T) 	46.07 (Sig.	P≥.001)	Time:  4 <sup>a</sup> > 24 <sup>b</sup>	
A x T   1 By wt., 2 9	7.78 (Sig. 2 % n = 2	$P \ge .022)$ NB = NC	 -  > break	

Table 5. Break force (g) of squid gels containing different protein adjuncts formed and heat-set after varying holding times at  $4^{\circ}$ C.

Treatment means in a column and level means in a row with same exponent letter did not vary significantly (P=.05)

va.			
Holding Time (hrs)	Protein Adjunct <sup>2</sup>	Mean <sup>3</sup>	S.D.
4	  Egg White (EW)  Soy Isolate (SPI)  Control (Cont)  Sodium Caseinate (Cas)	$13.05^{ab} \\ 14.00^{ab} \\ 11.93^{a} \\ 13.22^{ab}$	1.16 .71 .57 .49
24	  Egg White  Soy Isolate  Control  Sodium Caseinate   Analysis of Variance: Fa	15.23 <sup>b</sup> 16.29 <sup>b</sup> 15.02 <sup>ab</sup> 15.90 <sup>b</sup> 	1.18 2.57 2.05 1.13
	F-value	Ranking of Le	evel Means
Adjunct (A) Time (T)	.99 (NS P≤.05) 13.21 (Sig. P≥.006)	  Adjunct:  SPI > Cas > EW  Time:  24 <sup>a</sup> > 4 <sup>b</sup>	> Cont
АхТ	.17 (NS P≤.05)	24 > 4	
Portion (%) compress By wt., 2 % Treatment mea exponen	of moisture in sample ex sion. $3_n = 2$ ans in a column and level t letter did not vary sig	pressed after 90 means in a row nificantly (P=.0	with same

Table 6. Expressible moisture<sup>1</sup> of squid gels containing different protein adjuncts formed and heat-set after varying holding times at 4<sup>0</sup>C.

Holding Time (hrs)	Protein Adjunct <sup>1</sup>	Mean <sup>2</sup>	S.D.
4	  Egg White (EW)  Soy Isolate (SPI)  Control (Cont)  Sodium Caseinate (Cas)	74.14 <sup>C</sup> 73.18 <sup>abc</sup> 73.79 <sup>bc</sup> 71.76 <sup>a</sup>	.86 .57 1.37 .38
24	  Egg White  Soy Isolate  Control  Sodium Caseinate	$73.68^{bC} 73.31^{abC} 74.31^{C} 72.06^{ab} $	.71 .69 .75 .58
Analysis of Variance: Factorial Design			
<b></b>	F-value	Ranking of Le	evel Means
  Adjunct (A)    Time (T) 	6.15 (Sig. P≥.018) .10 (NS P≤.05)	Adjunct:  Cont <sup>a</sup> > EW <sup>a</sup> > SP1  Time:  24 > 4	a> Cas <sup>b</sup>
A x T	.28 (NS P≤.05)	 !	
1 Bv wt., 2	$\frac{2}{n} = 2$		

Table 7.	Moisture content	(%) of	squid gels containing different
	protein adjuncts	formed	and heat-set after varying
	holding times at	4 <sup>0</sup> C.	

Treatment means in a column and level means in a row with same exponent letter did not vary significantly (P=.05)
Table 8. Coo pro ho:	ok-cool loss <sup>1</sup> of squid ge otein adjuncts formed and lding times at 4 <sup>0</sup> C.	ls containing di heat-set after	fferent varying
Holding			
Time (hrs)	Protein Adjunct <sup>2</sup>	Mean <sup>3</sup>	S.D.
4	Egg White (EW)	5.29 <sup>a</sup>	. 66
	Soy Isolate (SPI)	12.41 <sup>bC</sup>	. 17
	Control (Cont)	16.65 <sup>Cu</sup>	3.82
	Sodium Caseinate (Cas)	19.75 <sup>0</sup>	2.42
24	Egg White	4.74 <sup>a</sup>	. 42
	Sov Isolate	10.70 <sup>D</sup>	. 28
	Control	14.70 <sup>bce</sup>	2.28
	Sodium Caseinate	18.25 <sup>de</sup>	2.84
	Analysis of Variance: Fa	 ctorial Design	
	F-value	   Ranking of Le	vel Means
Adjunct (A)	33.75 (Sig. P≥.001)	  Adjunct:  Cas <sup>a</sup> > Cont <sup>a</sup> > SP	
Time (T)	1.90 (NS $P \le .05$ )	Time:  A > 24	
АхТ	.37 (NS P <u>&lt;</u> .05)		
Portion (%) By wt., 2 %	of raw weight lost durin n = 2	g cooking	

Treatment means in a column and level means in a row with same exponent letter did not vary significantly (P=.05)

ho	lding time	es at 4 <sup>0</sup> C.	1	· · ·		
Holding   Time   (hrs)		Protein Adjunct <sup>2</sup>	     Mean <sup>3</sup>	S.D.		
4	Egg White	e (EW)	9.65	.64		
1	Soy Isola	ate (SPI)	10.05	.49		
	Control	(Cont)	10.30	1.13		
1	Sodium Ca	aseinate (Cas)	NB			
24	Egg White	e	9.85	.21		
1	Soy Isola	ate	9.95	.07		
1	Control		9.80	.14		
ļ	Sodium Ca	aseinate	NB	8		
	Analysis	of Variance: Fa	ctorial Design			
		F-value	Ranking of L	evel Means		
  Adjunct (A)	.31 (NS	P <u>≤</u> .05)	Adjunct:			
Time (T)	.05 (NS	P<.05)	Time:			
		,	4 > 24			
AXT	.37 (NS	P <u>≤</u> .05)				
Distance (mm) sample is compressed at gel break						
<sup>-</sup> By wt., 2 %	~n =	reak				

Table	9.	Deformat	tion <sup>1</sup> d	of	squid	gels	containing	g diffe	erent
		protein holding	adjuno times	cts at	forme 4 <sup>0</sup> C.	ed and	i heat-set	after	varying

### Preparation and Evaluation of a Restructured Squid Product

## Preparation of Restructured Squid Product

The common method of manually cleaning squid (Loligo opalesence) yielded a total of  $52\pm2.4$  % edible flesh composed of skinned mantles (70 %) and skin-on tentacles (30 %) (Table 10). The yield after pressing (dewatering) dropped to  $33.6\pm4.5$  % based upon round weight. Most of the press loss was comprised of water and soluble proteins that were pressed from the mince, however, operational losses (unrecovered squid flesh that adhered to the cheese cloth) also contributed to a lesser degree. The raw yield was increased slightly to 36.5 % when dry ingredients (8.5 %) were incorporated.

The proper combination of gel sol carrier and fibers was critical to successful forming of the mixture into a restructured product. The mixture was excessively adhesive and clogged the forming machine when it contained >40 % carrier. Less than 40 % carrier, provided insufficient binding of the fibers and produced a formed product with a crumbly texture. A mixture of 60 % fibers and 40 % carrier produced efficient molding with the forming machine. This proportion also provided adequate binding to maintain the integrity of the test product (as judged visually).

In order to allow for cook-cool losses, 45 % of the total amount of paste was extruded into long strands and heat set. Paste formulations lost an estimated 3 % when steam cooked and cooled in the extruded form. The gelled fibers were mixed with the carrier and formed into the restructured product with a yield

of 33.4 % based upon round weight. The influence of different protein adjuncts on cook-cool loss that was seen in gels prepared in the plastic trays, was not observed in the test product. The water holding properties of added potato starch

(5 %) appeared to mask the effects of the protein adjuncts (2 %).

Table 10. Processing yields for restructured squid product.

Process Component	Yield
Tentacles, cleaned <sup>1</sup>	15.3 <u>+</u> 1.03
Mantles, cleaned <sup>2</sup>	37.5 <u>+</u> 1.38
Total edible flesh <sup>3</sup>	52.2 <u>+</u> 2.39
Pressed flesh <sup>4</sup>	33.6 <u>+</u> 4.50
Press loss <sup>5</sup>	35.6 <u>+</u> 4.43
Cook-cool loss <sup>6</sup>	3.0 <u>+</u> 0.42
Final product <sup>7</sup>	33.4 <u>+</u> 0.95

<sup>1</sup>Skin-on, beaks and adhering flesh removed; % by weight based on whole squid <sup>2</sup>Eviscerated, skin-off, fins included; % by weight based on whole squid <sup>3</sup>Cleaned mantles and tentacles; % by weight based on whole squid <sup>4</sup>Percent by weight based on whole squid <sup>5</sup>Percent difference between weight of minced squid before and after pressing

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<sup>6</sup>Percent difference between weight of extruded strands before
and after cooking
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<sup>7</sup>Percent by weight based on whole squid
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n=15 for all yields except cook-cool loss and final product where n=3.

# Composition of Raw Squid and Restructured Squid Product

The moisture content of tentacle tissue with skin had a significantly (p>.05) higher moisture content than the mantle tissue without skin (84.8 and 83.2 %, respectively) (Table 11).

The difference is most likely attributed to water associated with the skin.

	Mantle <sup>2</sup>	Tentacle <sup>3</sup>	Product <sup>4</sup>
Moisture	83.20 <u>+</u> 0.27	84.80 <u>+</u> 0.25	71.80 ± 0.47
Protein	12.29 <u>+</u> 0.18	10.86 <u>+</u> 0.16	18.34 <u>+</u> 0.29
   Lipid	1.51 <u>+</u> 0.03	1.78 <u>+</u> 0.07	2.48 <u>+</u> 0.09
NPN <sup>5</sup>	0.30 <u>+</u> 0.02	0.23 <u>+</u> 0.03	0.13 <u>+</u> 0.01
   Ash	1.07 <u>+</u> 0.05	0.81 <u>+</u> 0.07	2.32 <u>+</u> 0.02
Сно <sup>6</sup>	Trace	Trace	4.94 ± 0.84

Table 11. Composition<sup>1</sup> of raw edible squid muscle and restructured squid product.

Percent by weight on wet a basis; n = 3

<sup>2</sup> Without skin

<sup>3</sup> With skin

<sup>4</sup> Pooled sample of raw squid product containing different protein 5 adjuncts

Non protein nitrogen

° Carbohydrate

Pressing the squid meat and the addition of dry components (8.5 %) reduced the moisture content of the test product (71.8 %) from that of the raw squid meat. As a result of pressing and the addition of 2 % protein solids, the protein content of the test product (18.34 %) was approximately 58 % higher than that of the raw squid (11.58 %). The inclusion of edible lubricating oil from the forming machine may be partially responsible for the higher lipid content in the test product. The carbohydrate content was neligible in the raw squid, but approximated 5 % in the final product due to the addition of potato starch.

## Sensory Evaluation of Restructured Squid Product

<u>Consumer Preference Panel</u> The type of protein adjunct added to squid gels had no affect (P>.05) on the mean preference scores for all the sensory attributes evaluated (Table 12). Ranking of treatment mean scores based upon non-significant differences (P=.05) did not reveal a clear trend for a preference of a specific adjunct. Preferences for adjuncts varied with respect to individual sensory factors. On a nine point hedonic scale the restructured squid product received a mean score of 6.00 or better for flavor,

different protein adjuncts.							
-   	Protein Adjuncts <sup>2</sup>						
Attribute	Egg White (EW)	Soy Protein (SPI)	Sodium Caseinate   (Cas)				
Texture	6.37 <u>+</u> 1.54	6.43 <u>+</u> 1.38	6.37 <u>+</u> 1.72				
Flavor	6.31 <u>+</u> 1.86	6.09 <u>+</u> 1.65	6.14 <u>+</u> 1.63				
Appearance	6.51 <u>+</u> 1.69	6.60 <u>+</u> 1.22	6.63 <u>+</u> 1.57				
Seafood Flavor	5.06 <u>+</u> 1.73	4.77 <u>+</u> 1.78	4.40 <u>+</u> 1.72				
  Desirability	5.91 <u>+</u> 1.60	5.83 ± 1.62	6.17 <u>+</u> 1.42				

Table 12. Mean<sup>1</sup> consumer preference scores for various sensory attributes of a restructured squid product containing different protein adjuncts.

Attribute	F value			Ranking of Treatment Means
Texture	0.02	NS	P≤.05	SPI > EW = Cas
Flavor	0.17	NS	P<.05	EW> Cas> SPI
Appearance	0.05	NS	P<.05	Cas> SPI> EW
Seafood Flavor	1.25	NS	P<.05	EW> SPI> Cas
Desirability	0.46	NS	P<.05	Cas> EW> SPI

Analysis of Variance

<sup>1</sup>n=35 <sup>2</sup>By weight, 2 %

Score range: 1, extremely undesirable to 9, extremely desirable

texture, appearance, and overall desirability, indicating they were slightly desirable.

<u>Trained Panel</u> Mean scores by trained sensory judges revealed no significant effect ( $P \le .05$ ) of protein adjunct on perceived cohesiveness, juiciness, and chewiness of the restructured squid product (Table 13). The firmness attribute was found to be significantly influenced ( $P \ge .02$ ) by the protein adjuncts incorporated into the product. The mean firmness score for squid products containing soy protein isolate and egg white were superior (P = .05) to those incorporating sodium caseinate. The firmness scores for products containing soy protein isolate and egg white were equal (P = .05).

Table 13. Mean<sup>1</sup> trained panel magnitude estimation scores for various sensory attributes of restructured squid products containing different protein adjuncts.

	Protein Adjuncts <sup>2</sup>						
Attribute	Egg White (EW)			Soy Protein (SPI)	Sodium Caseinate   (Cas)		
Firmess	1.06 ± 0.16			09 <u>+</u> 0.13	0.89 ± 0.13		
  Cohesiveness	1.00 <u>+</u> 0.23		1	.16 <u>+</u> 0.18	0.96 ± 0.37		
Juciness	1.03 ± 0.26			).90 <u>+</u> 0.28	1.18 <u>+</u> 0.14		
Chewiness	30.88 <u>+</u> 7.24   3		32	2.25 <u>+</u> 6.11	   26.00 <u>+</u> 8.28		
	Analy	sis of	Vari	ance	, , , , , , , , , , , , , , , , , , ,		
Attribute	F-value		Ranking of	Treatment Means			
Firmess	4.70 Sig P>.02			$SPI^{a} > EW^{a} > Cas^{b}$			
Cohesiveness	1.14 N	S P<.	<b>0</b> 5	SPI>	EW> Cas		
Juciness	2.97 N	5 P <del>&lt;</del> .	05 İ	Cas>	EW> SPI		
Chewiness	1.63 N	$S P \leq .$	05	SPI>	EW> Cas		
l ——							

<sup>1</sup> By weight, 2 % <sup>2</sup>n=8

Treatment means with the same exponent letters in the same row did not vary (P=0.05). Although texture measurements were carried out on gels that did not contain potato starch, sensory scores for the firmness of the restructured squid product were consistent with measurements of break force. Break force could not be measured for squid gels containing sodium caseinate due to their lack of a break point when compressed. The trained sensory panel perceived the squid product containing sodium caseinate to be the least firm. Break force values for squid gels containing egg white and soy protein isolate did not vary (P=.05). The trained sensory panel also found no difference in the firmness of the evaluated products containing either egg white or soy protein isolate.

#### SUMMARY AND CONCLUSIONS

The raw and cooked pH of minced squid was increased from 6.2 to 7.7 and 6.4 to 8.3, respectively, by the addition of 0.75 % sodium carbonate. Incremental additions of sodium carbonate totaling 0.5 % or less in minced squid were more effective for raising pH than levels totaling greater than 0.5 %. As observed for red meat systems, heat affected an increase in the pH of minced squid. Cook-cool loss and gel break force decreased linearly with respect to incremental additions of sodium carbonate and elevating pH. Conversely, gel moisture content increased in a linear manner.

The higher pH of squid muscle systems promoted an increased hydration of the polypeptide chains and more water was retained within the protein network structure. The decrease in break force and cook-cool loss observed was a direct result of the increased moisture holding capacity of the squid gels.

Holding squid gel sols at  $4^{\circ}$ C for 24 hours before forming and heat setting had a significant effect on the break force (P $\geq$ .001) and expressible moisture content (P $\geq$ .006) of squid gels containing different protein adjuncts. Holding time had no significant effect (P $\leq$ .05) on gel moisture content, cook-cool loss or the deformation occurring at break. A significant (P $\geq$ .022) interaction of protein adjunct and holding time was observed for break force measurements, but not for expressible moisture, cook-cool loss or deformation occurring at break.

Holding sols at  $4^{\circ}$ C for 24 hours caused a significant reduction (P>.05) in the break force values for the control squid

gels and the gels containing soy protein isolate. The holding treatment did not have a significant effect ( $P \ge .05$ ) on the break force values for squid gels containing egg white.

Three mechanisms were hypothesized to have mediated these results, either individually or in combination. Autolytic proteolysis of myosin during holding could have occurred and reduced gel forming ability. Protease inhibitors in egg white retarded proteolytic activity and the functionality of proteins critical for gel formation was preserved. Gel sols may have underwent cold temperature setting (suwari) during the 24 hour holding period at 4<sup>0</sup>C. Suwari formation prior to heat setting produces gels that are superior in strength to gels formed directly from sols without the preliminary suwari step. However, if the structure of the suwari gels are disrupted before heat setting, the resulting gels are weaker. Reactive sulfhydryl groups on the squid proteins may have been oxidized during the holding period. These reactive groups are essential for the formation of disulfide bonds during heat-setting; without reactive sulfhydryl functions, a strong gel would not have formed. The addition of egg white to the system contributed an appreciable quantity of protein rich in sulfhydral functions. The interaction of egg white and squid muscle proteins could have combined to strengthen the protein network structure of the gel.

Protein adjuncts had a significant effect on the break force, moisture content, and cook-cool loss ( $P \ge .026$ , .018, and .001, respectively). Protein adjunct did not have a significant effect ( $P \le .05$ ) on expressible moisture or deformation at break.

Squid gels containing sodium caseinate did not fracture when compressed. The break force of squid gels containing sodium caseinate could not be measured because the gels were smashed in one smooth motion without any indication of fracture on the force time curve. The mean break force of the control squid gels was significantly (P=.05) greater than gels containing soy protein isolate or egg white. The latter possessed equal gel strengths (P=.05).

The mean moisture content of the squid gels containing sodium caseinate was significantly (P=.05) less than the control gel or gels containing either egg white or soy protein isolate. Control and squid gels containing egg white and soy protein isolate possessed equal (P=.05) moisture contents.

Squid gels containing sodium caseinate lost the most weight through cooking and cooling (19.8 %), but did not vary (P=.05) from the control (16.7 %). The cook-cool loss of squid gels containing soy protein isolate (12.4 %) were lower (P=.05) than either the control or the gel containing sodium caseinate. Gels containing egg white lost the least (P=.05) weight (5.3 %) through cooking and cooling.

Processing squid yielded 52.2 % cleaned edible flesh (mantles and tentacles) based upon the round weight. A yield of 33.4 % restructured squid product based upon round weight was observed. A mixture of three parts of fibers prepared from heat-set gel to two parts carrier (freshly prepared gel sol) was found to mechanically form most efficiently.

The consumer preference panel detected no significant ( $P \le .05$ ) differences between the various protein adjuncts incorporated into the restructured product. The effects of protein adjuncts on gel texture that were demonstrated through objective measures, were more obscure in the restructured squid product. The samples that were subjected to objective measurements did not contain potato starch whereas the final restructured products did. The dominating textural influence of 5 % potato starch in the restructured product probably concealed the textural influence of the protein adjuncts. The restructured product received a mean score of at least 6 on a 9 point hedonic scale for texture, flavor, appearance, and overall desirability. Judges perceived the product as being slightly desirable.

Firmness was the only sensory attribute that a trained sensory panel found to be significantly ( $P \ge .02$ ) influenced by incorporated protein adjuncts. The product containing sodium caseinate received a lower (P=.05) rating for firmness than products containing either egg white or soy protein isolate. The sensory panel scores for firmness appeared to be consistent with the measurements for break force.

Further research is required to elucidate the mechanism responsible for the significant loss in gel forming ability that resulted from storage of squid protein sols at 4<sup>o</sup>C for 24 hours before forming and heat setting. The effects of other binders and gelling agents such as cellulose, gums, and modified starches on the texture of squid gels should be investigated to further expand the potential for processing squid into consumer oriented products.

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