Section I: Literature Review

1. Squid

Squid or calamari is a cephalopod which is one of the three groups of mollusks: (1) univalves having a single shell; (2) bivalves having two shells; and (3) cephalopods. There are almost 1000 species of cephalopods, however, the few species commercially caught are squid, cuttlefish, and octopus. Cephalopods are marketed in various forms which include fresh, frozen, canned, dried, salted, and smoked. The most commercially important group of all cephalopods is squid. The primary exploited species of north Atlantic squid are the Atlantic short-finned squid (*Illex illecebrosus*) and the Atlantic long-finned squid (*Loligo pealei*) which belong to the Ommastrephidae and Loliginidae families respectively.

1.1 North Atlantic long-finned squid (*Loligo pealei*)

North Atlantic long-finned squid is also known as winter squid, Boston squid, bone squid, and trap squid (Sea Grant). Long-finned squid inhabit inshore waters and are caught in the Spring and early Summer months when the squid return from the continental shelf for feeding and spawning. The long-finned squid has a broad pen and long fins which are about half of the mantle length as shown in Fig. 1. Normally, the

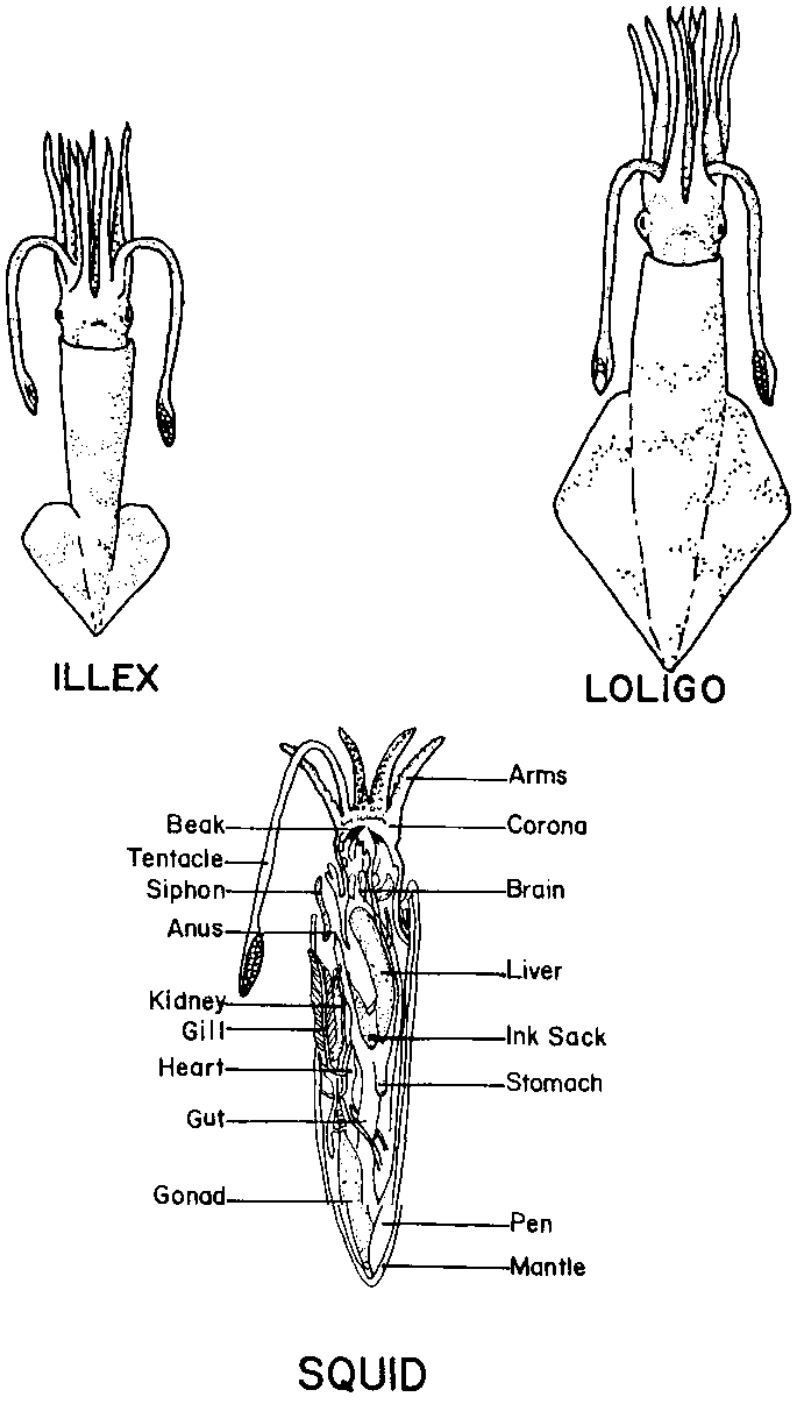


Fig. 1: The Structure and Internal Organs of Squid (*Illex sp. and Loligo sp.*) (Sea Grant).

41long-finned squid is larger and has a thicker mantle wall than the short-finned squid. Its market acceptability is also greater due to its texture which is considered as less rubbery.

1.2 North Atlantic short-finned squid (*Illex illecebrosus*)

Other names of north Atlantic short-finned squid are Summer squid and bait squid (Sea Grant). Short-finned squid is an oceanic, not an inshore species as long-finned squid, and therefore can be caught on the continental shelf during the summer season. Short-finned squid has a slender pen and shorter fins than long-finned squid. The fin length is only one third of the mantle length as shown in Fig. 1. The short-finned squid has a smaller size and thinner mantle wall than the long-finned squid but a more leathery texture which negatively affects its consumer acceptability.

1.3 The structure of squid

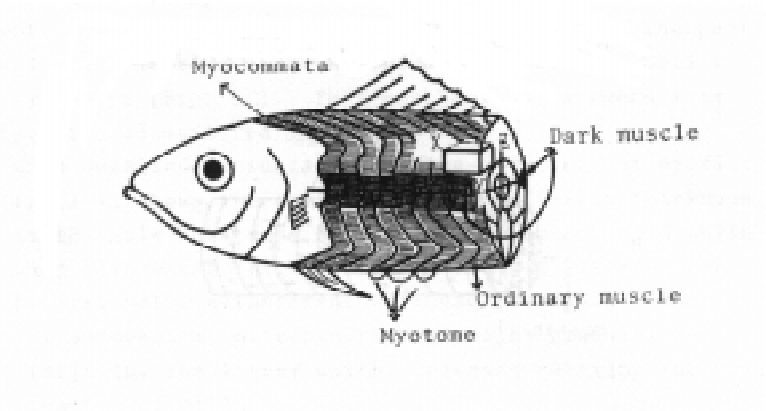
The squid body is usually called the mantle or torso (in Japan). Squid has no visible shell like univalves and bivalves but it contains the chitinous cartilage pen or quill in the inner mantle. The surface containing the pen and fins is the dorsal surface while ventral surface contains the funnel. The color of the dorsal surface is milky white and is darker than the ventral because of the higher amount of chromatophore. Chromatophore or pigment cells contain red, blue, yellow, and black pigments varying upon species. Squid have 10 appendages or 5 pairs of appendages around the head.

Eight or 4 pairs are arms which are short and heavy and the other two are tentacles which are lighter and twice longer than the arms (Fig. 1). Squid meat reddens when the autolytic process occurs. Autolysis causes the rupture of pigment cells which produce a reddening of the meat.

The muscle of the squid mantle differs from the muscle of fish and mammals. The muscle fibers in fish muscles run parallel to the long axis of the fish as shown in Fig. 2. Contrary, the muscle fibers of the squid mantle are arranged orthogonally into layers of the radial (perpendicular to the skin) and the circumferential band (parallel to the skin) sandwiched between the outer and inner tunic of the connective tissue (Otwell and Hamann, 1979). The fibers of the connective tissues in the outer tunic are arranged more orderly than the inner tunic. The outer and inner tunics are covered again by the outer lining and a nonfibrous visceral lining respectively (Fig. 3). Sometimes, the muscle running perpendicular to the skin is called muscle B while the muscle which runs parallel to the skin is called muscle A (Sugiyama et al., 1989) as illustrated in Fig. 4.

1.4 The chemical composition

The proximate composition of squid meat is 75-84% water, 13-22% crude protein, 0.1-2.7% lipids, and 0.9-1.9% minerals (Sikorski and Kolodziejska, 1986) and is similar to that of white-meat fish (Sugiyama et al., 1989). The sarcoplasmic protein accounts for 15% of the total protein (Table 1) and consists of various types of proteins, most having enzymic activity. One example is proteinase, which causes degradation of



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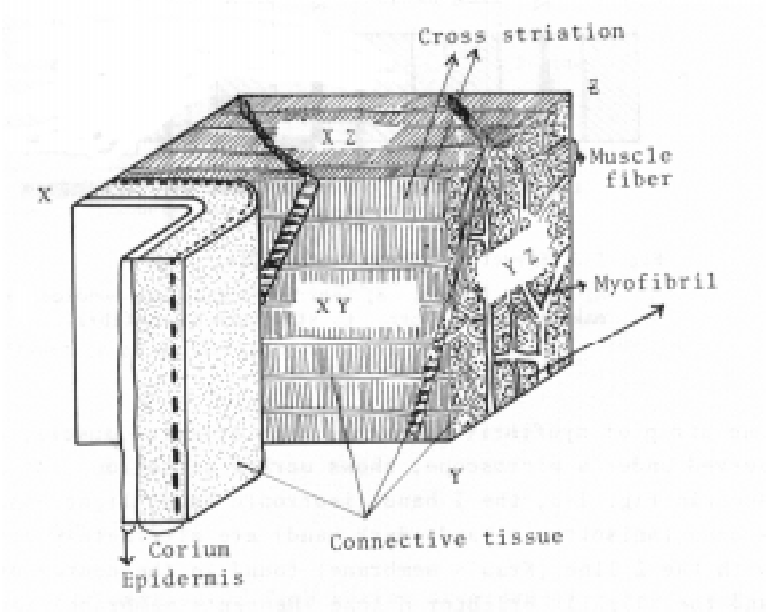


(b)

Fig. 2: The tissue structure of fish (T. japonica) (Sugiyama et al., 1989)
(a) Direction of muscle when skin is peeled
(b) The enlargement of XYZ section in Fig. (a)

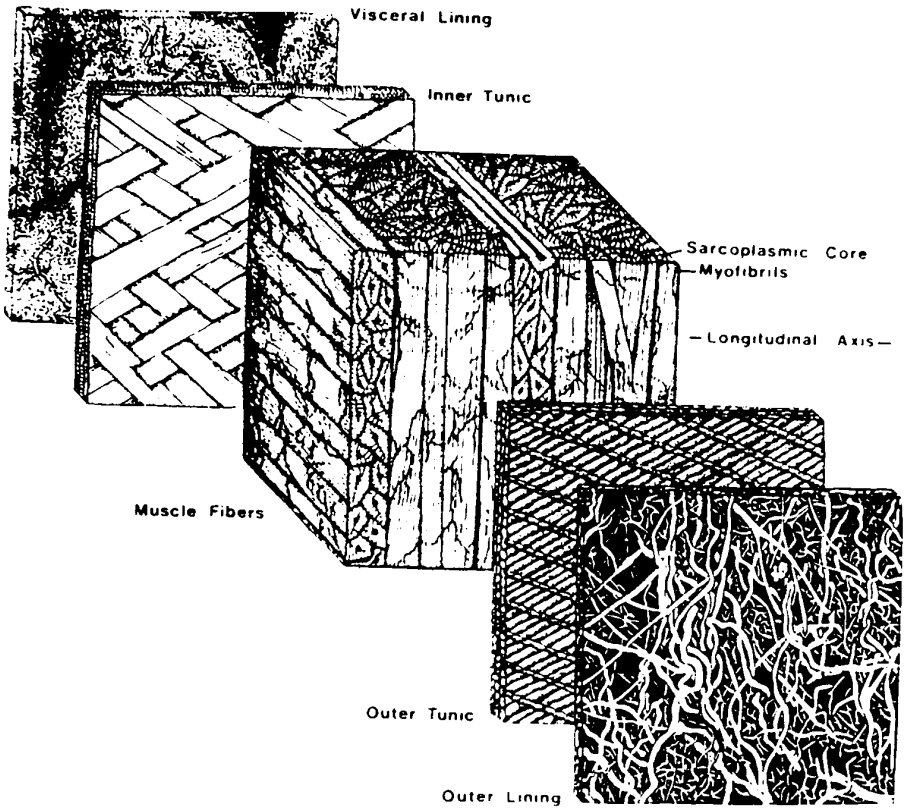


Fig. 3: Tissue composition in the squid mantle *(Loligo pealei)* in the view of a cube cut from the entire thickness of the mantle. Skin has been removed from the outer lining. The longitudinal axis refers to the head to tail axis of the squid mantle (Otwell and Giddings, 1980)

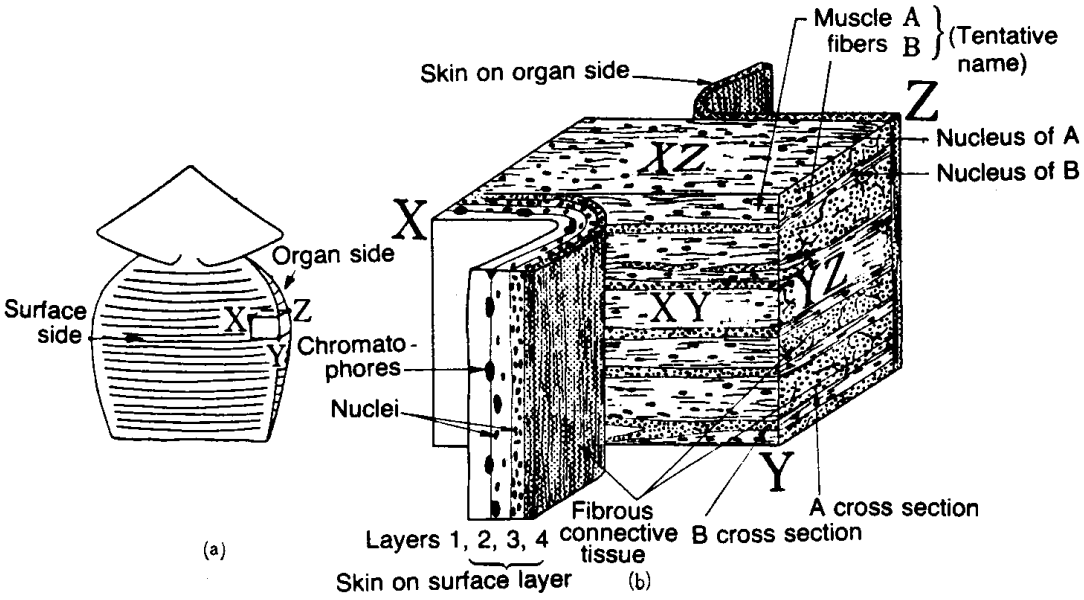


Fig. 4: The tissue structure of squid mantle (*T.pacificus*) (Sugiyama et al., 1989)

(a) Direction of muscles when abdomen is open and skin has been removed

(b) The enlargement XYZ section of the cube cut from the entire thickness of mantle in Fig. (a)

Table 1: Protein and Non-Protein Nitrogen in Squid Meat (Sikorski and Kolodziejska, 1986)

Species and body part	Total protein nitrogen (gN/100g)	Protein fractions' nitrogen in total protein (%)			Non-protein N in total nitrogen (%)
		Myofibrillar	Sarcoplasmic	Collagen	
Illex, tentacles	1.95	64.8	15.2	16.0	38.8
Illex, mantles	2.01	74.6	11.5	11.1	38.1
Loligo, mantle	1.85	79.0	14.9	3.0	36.2

myofibrillar proteins. *Illex* posses both acid and alkaline proteases over a pH range of 2.6 to 7.4 with the optimal pH of 3.0, 5.8, and 6.6. The protease activity of fresh *Illex illecebrosus* at pH 3 and pH 6.6 is several times higher than that of fresh flounder and frozen red hake. The protease activity in frozen tissue of *Illex illecebrosus* measured at pH 6.6 is also reported higher than that of frozen *Loligo pealei* (Stanley and Hultin, 1984).

Myofibrillar proteins constitute about 76% of the total protein content and differ from that of fish and mammals by being more water-soluble. Approximately 85% of the total protein in squid muscle will dissolve in water.

The collagen in the squid muscle is somewhat higher than that of fish muscle. The content of collagen in fish muscle ranges from about 1 to 12% of the crude protein. In the mantles of *Loligo* and *Illex*, the amount of collagen is about 3% and 11% respectively. The different amount of collagen in squid mantles varies with the squid species and is presumably responsible for its tough texture. The high amount of collagen in squid as compared to fish is due to the structure as described above (Sikorski and Kolodziejska, 1986).

2. Engineered foods

2.1 Definition

Engineered foods, fabricated foods, restructured foods, structured foods, designed foods, architecturally created foods, and food analogues are terms used to

described foods prepared from individual ingredients or components according to predesigned plans to yield finished products having specified physical, chemical, and functional properties (Glicksman, 1985). The terms also apply to food products prepared by any method which reshape food pieces or particles into a larger and more appealing form (Anon, 1983).

2.2 History/Background

The production of fabricated foods generally results from consumer demands for convenience, nutritional composition improvement, undesirable components removing, food resource extending, costly food imitation, new processing technology requirement, new food resource utilization, by-product or waste materials utilization, cost and economy improvement, new variety creation, preservation and functional utility improvement. Fabricated foods originated in the early 1940s during World War II. In the 1940s and 1950s, the lack of time for food preparation caused by war demands initiated the production of timesaving and convenience foods.

The 1950s and 1960s contained high numbers of individuals consuming poor quality protein foods. Consequently, protein-based beverages, cereals, and pastas were fabricated throughout the world in an attempt to alleviate this problem.

In the 1970s, a shortage of all types of food occurred. Many food innovations, such as high-yield grains, new food plants, and aquaculture, were developed to increase food quantity. Meat analogues and substitutes were developed to address the soaring price of meat as a result of high petroleum costs which were controlled by OPEC.

However, meat analogue development was curtailed or stopped after new oil resources were found and petroleum prices were lowered.

In the 1980s, meat analogues and fabricated meat chunks were first successfully developed by R.A. Boyer (Glicksman, 1985). Meat analogues were converted from vegetable proteins, such as soy proteins, by solubilizing the protein, unfolding the protein chains, reforming or simulating muscle protein fibers, and spinning fibers to the desirable meat-like structure. This process known as the Boyer process, was used to fabricated meat, fish and poultry-like products, such as chicken chunks (Glicksman, 1985). The concept of restructuring meat and poultry products had been developed since the last decade from lean trimmings and cuts. Restructured steak provided a low-cost, uniform product that imitated fresh muscle in flavor, color, and textural properties. Other types of restructured products, including roasts, chops, cutlets, strips, and cubes, were also produced through the same technology and were well received in the marketplace (Mandigo, 1986).

The seafood industry imported surimi technology from Japan to produce restructured seafood closely resembling the flavor, appearance, texture, and shape of natural seafoods such as crab legs, scallops, and lobster tails (Glicksman, 1985). Recently, new technology in the binding process, such as the cold-set binder process, was introduced into the meat industry. Cold-set binders, which include alginate systems, Pearl Meat Binders® and Fibrimex®, are used in restructured meats to hold pieces together without the application of a thermal process (Esguerra, 1995).

3. Hydrocolloids or gums

Gums are long-chain polymers, primarily carbohydrates, that are soluble or disperse in water. Gum particles are suspended in solution as colloids, therefore, gums are also known as hydrocolloids. Gums are effective as binding and texturing agents and provide structure, functionality, and desirable properties to fabricated foods. The important functionality of gums are viscosity or thickening, gelling, and freeze-thaw stabilization properties (Lee, 1997). The common edible hydrocolloids used in food applications were categorized by Glicksman (1985) and are listed in Table 2.

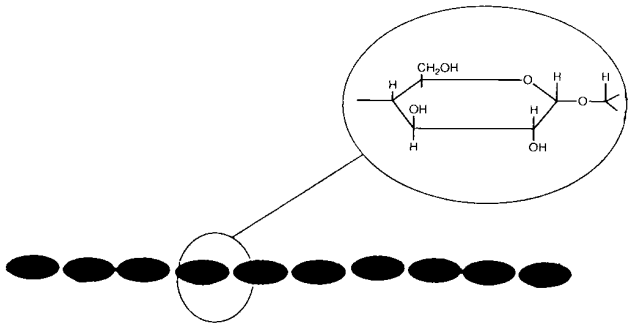
3.1 Starch

3.1.1 The structure of starch

Starch is composed of two types of molecules, amylopectin and amylose. Amylose is a predominantly linear molecule of D-glucose units ranging from 250 to 2000 units joined by α-1,4 linkages while amylopectin is a highly branched polymer of D-glucose units linked by α-1,4 and α-1,6 bonds every 20-26 monomer units as shown in Fig. 5. Amylose and amylopectin molecules are arranged radially and orderly within starch granules. The branches of amylopectin form both crystalline and amorphous regions in alternating layers with the amylose molecules occurring among the amylopectin molecules.

Table 2: The classification of common edible hydrocolloids (Glicksman, 1985)

Exudates	Extracts	Flours	Biosynthetic	Semi-synthetic	Synthetic
Plant:	Seaweed:	Seed:	Dextrans	Cellulose derivatives:	Polyvinylpyrrolidone(PVP)
Arabic	Agar	Locust bean	Xanthan	Carboxymethylcellulose	
Ghatti	Alginates	Tara	Curdlan	Methylcellulose	Polyethylene oxide
Karaya	Carrageenan	Tamarind	Polytran	Hydroxypropylcellulose	polymers (Polyox)
Tragacanth	Furcellaran	Quince	Gellan	Hydroxyethylcellulose	
		Psyllium seed	Pullalan	Hydroxypropylmethylcellulose	Acrylic acid polymer
	Plant:	Flax seed			(Carbopol)
	Pectin			Starch derivatives:	
	Arabinogalactan	Cereal starches:		Hydroxypropyl starch	Methl vinyl ether/maleic
		Corn			anhydride
	Animal:	Wheat		Propylene glycol alginate	
	Gelatin	Rye			Polyvinyl alcohol
		Waxy maize		Low methoxy pectin	
	Cereal:				Polyethylene glycol
	Corn hull	Tuber:		Hydroxypropyl guar	polymers
	Oat	Potato starch			
		Konjacmannan			
	Vegetable:				
	Okra	Root:			
		Tapioca starch			



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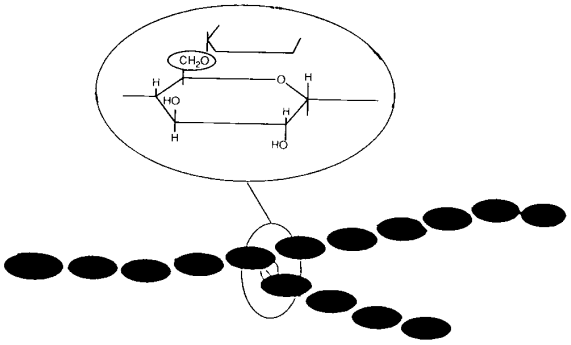


(b)

Fig. 5: The structure of starch (Imeson, 1992)
(a) amylose
(b) amylopectin

3.1.2 Functional properties of starch: gelatinization and gel formation

The major functional properties of starch are gelatinization and gel formation. When an aqueous starch solution is heated, the starch granules undergo the gelatinization process which is the disruption of molecular order within the granules. With continued heating, starch absorbs the surrounded water molecules resulting in the: swelling of granules; leaching of amylose; and eventual disruption of the molecules. When this final stage is reached, the process is called pasting. When hot starch paste is cooled, the dissolved materials become less soluble (referred to a retrogradation) and generally results in a viscoelastic, firm, rigid gel as depicted in Fig. 6.

3.1.3 Modification of starch

Native starches are usually modified either chemically or physically to improve some inherent properties such as freeze/thaw stability, water holding capacity, texture, mouthfeel, and shelf-life stability that are suitable for specific applications. Chemical modification involves cross-linking, substitution, thin-boiling or acid hydrolysis, and oxidation. Physical modification involves heat treatment to pregelatinize starch granules. A precook process preswells the starch granules so they have the ability to form a paste or thicken in cold water without the need for subsequent heating. Starch prepared from a physical modification procedure is usually called cold-water swelling starch or instant starch. The other specialty starch products used to a lesser extent are maltodextrins, corn syrup solids, dextrins, and cyclo-dextrins.

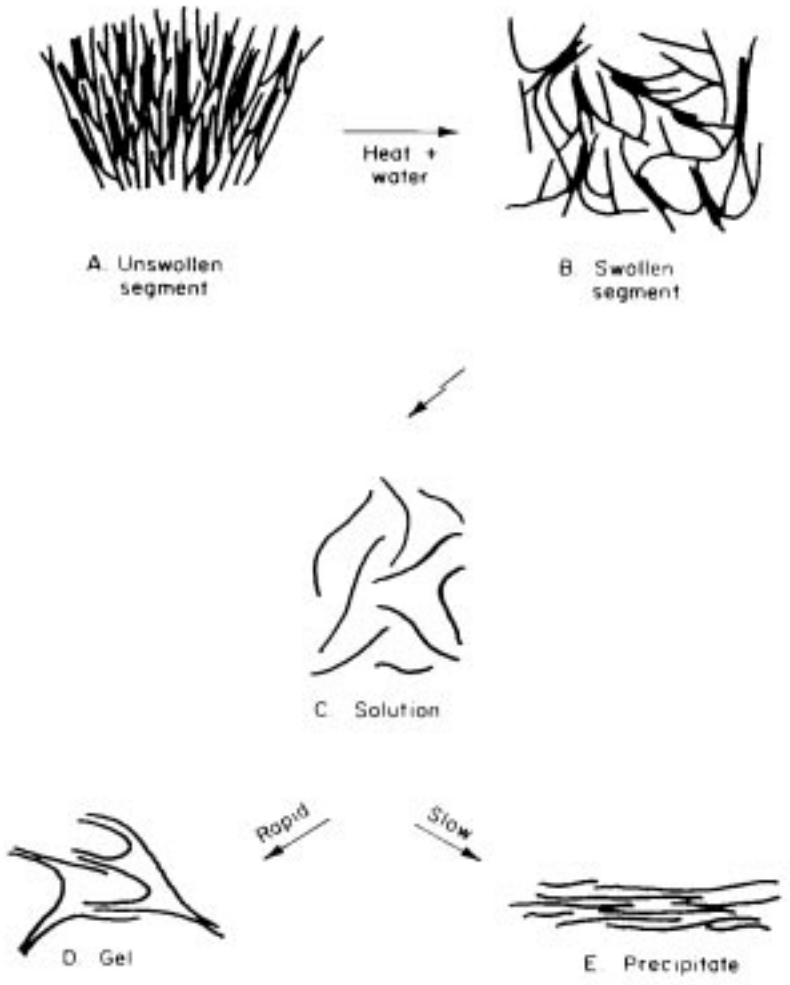


Fig. 6: The Gel Formation of Starch (Glicksman, 1969)

3.1.4 Application

The application of starch/modified starch in fabricated foods, such as shrimp, as a binder was reported by Soo and Sander (1977). Pregelatinized corn starch and corn flour combined with carboxymethylcellulose and xanthan gum (Hercules 30), originally formulated to enhance extruder performance and shape retention of onion rings, has been used as a binding-matrix agent. The product provided a significant decrease in cohesiveness and springiness of cooked patties than those made from isolate soy protein and isolate soy protein with Hercules 30 (pregelatininzed corn starch and corn flour combined with carboxymethylcellulose and xanthan gum).

Starch/modified starches are found in surimi and surimi-based products as a major ingredient to improve the texture, water-binding capacity, freeze/thaw stability, and for economic reasons (Kim and Lee, 1987). Also, a level of 5% or less of potato or wheat starch improved the textural properties of surimi gels. Starch increases gel strength and elasticity through both a composite reinforcing and water binding effect (Lee, 1984). The addition of starch, up to the level of 10%, tends to increase the cohesiveness (gel strength) and rigidity of products, while slightly decreasing the elasticity and firmness (Lee, 1986). The gel strength of surimi was reported to reach a maximum at 8% starch content (Chen et al., 1993). During heating, starch absorbs some water from the fish mince and becomes partially gelatinized and fills the pores of the protein network. The expansion of starch in the confined protein matrix generates pressure and as the protein matrix is withdrawn concomitant as the starch expands a firmer and slightly more cohesive gel matrix results (Lee et al., 1992). The gel

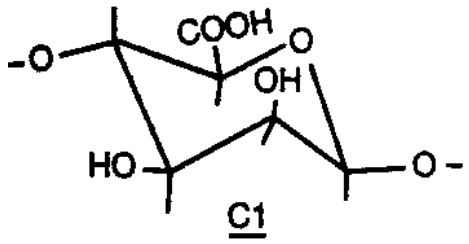
strengthening ability of starch is affected by its water-holding capacity during gelatinization and the viscosity of the gelatinized starch. The greater the water binding capacity and viscosity of starch, the greater is its gel strengthening ability (Lee, 1984).

The use of modified starch in surimi and surimi-based products is mainly for freeze-thaw stability. Modified starches are commercially prepared by hydroxypropylation or acetylaytion with or without crosslinking. Modified starches will increase freeze-thaw stability by preventing retrogradation or aggregation of amylose molecules with their cross-linked branches. The aggregation of amylose molecules results in the release of free water or freeze syneresis which is usually observed in unmodified starches**.** However, sometimes a freeze syneresis is desired to enhance moistness and juiciness of surimi. The recommended starch usage in surimi is half unmodified and half modified starch to access both the gel strengthening effect and freeze-thaw stability (Lee et al., 1992).

3.2 Seaweed extracts: alginate

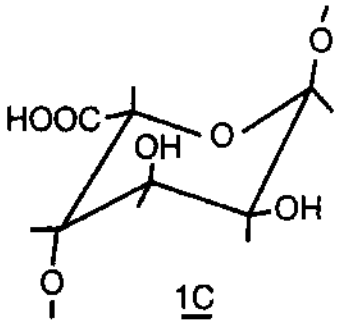
3.2.1 Chemistry of alginate

Alginates are the salts of alginic acids extracted from the brown seaweed (*Phaeophyceae*) and are a linear co-polymer of D-mannuronic and L-guluronic acid monomers as shown in Fig. 7 (Anon). These two monomers occur in homogeneous regions, M and G-blocks, and in a region of mixed units, MG-blocks (Fig. 8). The shape of polymannuronic acid is flat, ribbon-like whereas the shape of polyguluronic acid is



1,4 linked
β–D-mannuronic acid

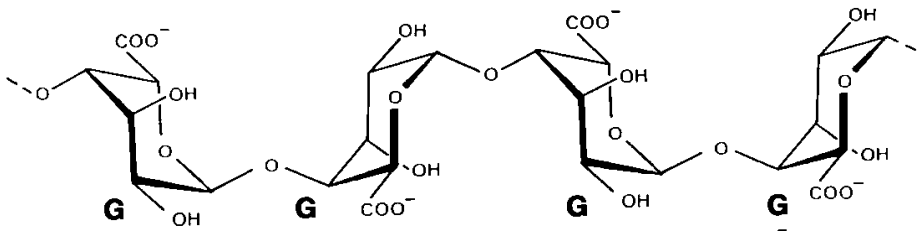
(a)



1,4 linked
α– L-guluronic acid

(b)

Fig. 7: The monomer of alginic acid (Anon)
(a) D-mannuronic acid monomer
(b) L-guluronic acid monomer



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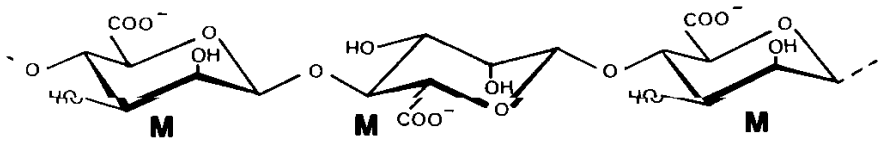

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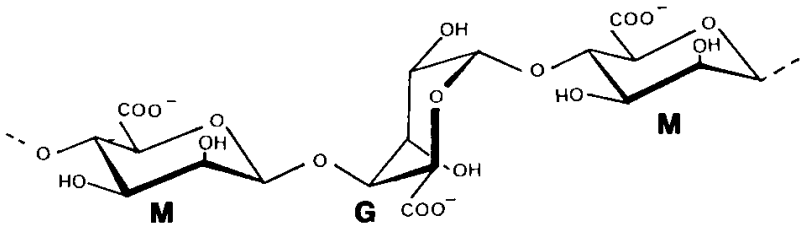

(c)

Fig. 8: The configuration of alginate block types (Imeson, 1992)

(a) homopolymeric G- blocks (GGGG)

(b) homopolymeric M- blocks (MMMM) and

(c) heteropolymeric blocks of alternating M and G (MGMGMG)

buckled, ribbon-like. Normally, alginic acid is insoluble and to make water-soluble products, alginic acids are transformed into commercial alginates through the incorporation of different salts (Fig. 9). The most widely used compound of alginic acid is sodium alginate (Na-alginate) which is readily soluble in hot or cold water (Imeson, 1992)

3.2.2 Functional properties of alginates: gel formation

One of the most important and useful properties of alginate in the food industry is the ability to form edible gels by reaction with calcium salt. Solutions of soluble alginate salts can form gels in the presence of calcium or other polyvalent metal ions. Alginate gel is of interest because once the gel is formed at room temperature, its shape and rheological characteristics will be retained throughout thermal processing and the gel network will remain through freeze and thaw cycles. Alginate gels are not thermoreversible as gelatin. The mechanism of alginate gelation is based on the reaction of the alginate molecule at G-block regions with calcium ions as shown in Fig. 10. The result is a junction zone which has been referred to as an “egg box” arrangement. The characteristic of the gel depends on the amount of calcium present in the system. With low levels of calcium, highly viscous and thixotropic solutions are obtained. At higher levels of calcium, precipitation or gelation occurs.

Another property of soluble alginate solution found useful in the food industry is film formation. Algin films can be prepared by drying a thin layer of

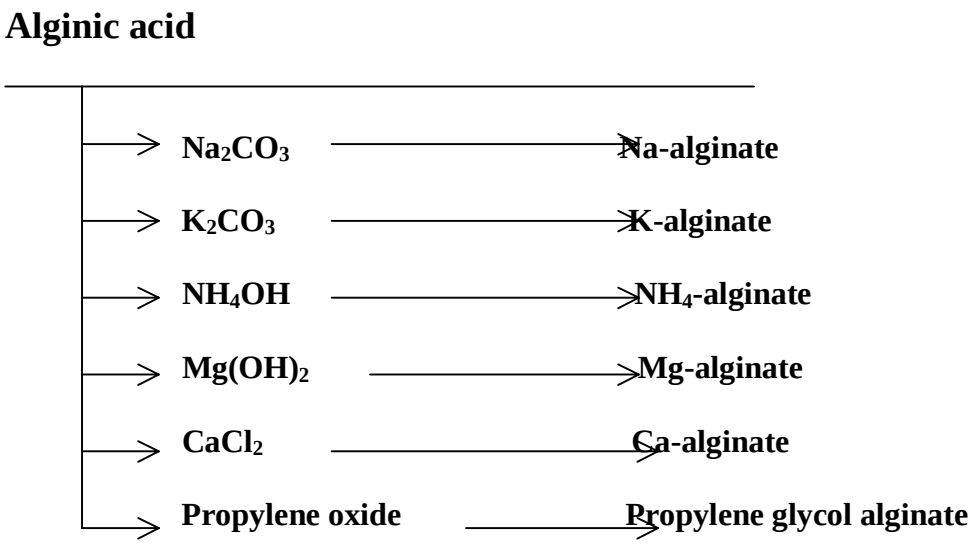

Fig. 9: The production of commercial alginates by incorporating different salts into alginic acids (Imeson, 1992)

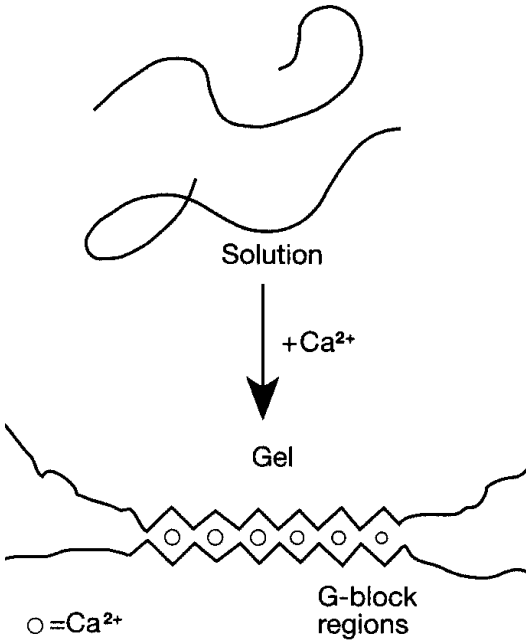

Fig. 10: The “egg-box” structure (Anon)

soluble alginate solution or by treating a soluble film with a di- or trivalent metal (Glicksman, 1985). Calcium ions are reported to be more effective than magnesium, manganese, aluminum, ferrous and ferric ions, in bridging alginate chains together by ionic interactions followed by interchain hydrogen bonding (Gannadios, 1997). Stronger alginate coatings will be obtained from calcium chloride rather than calcium gluconate, nitrate, and propionate.

3.2.3 The setting of algin/calcium gel

The method used to obtain alginate gels can be divided into three major methods: (1) diffusion setting; (2) internal setting; and (3) setting by cooling.

(1) Diffusion setting, the gel is set by slowly allowing calcium ions to diffuse into an alginate solution either spraying or immersing in the calcium salt bath. The reaction rate depends on the concentration of calcium in the setting bath. The most common source for calcium ions for diffusion is calcium chloride. Sometimes, calcium chloride is replaced by calcium lactate due to its unpleasant taste at high usage levels. This approach is useful for application on thin strips of material by providing a thin coating on product surfaces, such as onion rings, structured meat, and fish products. In the production of structured fish, algin was first dissolved in the water and then mixed with minced fish tissue. The fish paste was then formed into the desired shapes using molds. The surface of the mold was sprayed by a calcium chloride solution for initial setting. After the formed product is significantly strong, complete setting is executed in the setting bath.

(2) Internal setting, calcium is released under controlled conditions within the system. The reaction rate depends on the rate of calcium released to react within the alginates. The pH, amount, particle size, and intrinsic solubility characteristics of the calcium salt are the important factors affecting the releasing rate. The sources of calcium most commonly used are calcium sulfate, calcium sulfate dihydrate (gypsum), and calcium hydrogen orthophosphate (dicalcium phosphate). If the calcium-releasing rate is so rapid that premature gelation occurs, a calcium sequestrant can be used to control the reaction rate by competing with the alginate for calcium ions. Sequestrants previously used in food products are sodium hexametaphosphate, tetrasodium pyrophosphate, and sodium citrate. This method has been used to produce structured fruits, structured pet foods, and cold prepared desserts.

(3) Setting by cooling, alginate, calcium salt, and calcium sequestrant are dissolved in hot water and then allowed to set through cooling. The setting does not occur at high temperature despite the presence of both alginate and calcium in the system. The thermal energy of the alginate chains at high temperature is too high to permit the alignment of calcium. Water-based dessert gels, such as fruit pie fillings, is an example of a product produced through the setting cooling method (Anon).

3.2.4 Application

Alginates were first used in 1946 by Peschardt in fabricating fruit pieces and artificial cherries but the process was not successfully utilized until the 1970s. In the 1970s, with the renewed interest in eliminating waste, controlling pollution, and

reducing the high cost of fruit and vegetable ingredients, canned fabricated fruit pieces for baking applications were successfully introduced in the market.

Another early successful use of alginates was found in fabricated or pre-formed onion rings by using the waste from conventional onion processing. In this process, alginates in the matrix mix were combined with ground or diced fresh onion and then extruded into a ring form directly into a calcium salt bath to coagulate the onion ring. The fabricated onion rings were then battered, breaded and fried and usually stored frozen.

In 1986, alginates and calcium gel systems were tested as a raw and cooked binder in restructured beef steaks with satisfying results. The algin/calcium gel system can be used to bind the structured beef steaks in both the raw refrigerated and cooked state (Means and Schmidt, 1986). The effect of algin/calcium on the binding and sensory properties of structured beef steaks was subsequently studied. Products treated with algin/calcium showed better binding and color in the raw state, but lower palatability scores in the cooked state, than product made with salt/phosphate (Means et al., 1987). The level of algin and calcium allowed for use as binders in structured meat products was issued by the Food Safety and Inspection Service of the United States Department of Agriculture (USDA, FSIS, 1986) is a total of 1.5% sodium alginate, calcium carbonate, and lactic acid/calcium lactate. At a level of 0.57% combined algin/calcium binder (0.4% sodium alginate, 0.067% calcium carbonate and 0.1% lactic acid/calcium lactate), the desirable binding characteristics were achieved. However, the maximum level of 1.5%

may be considered as a greater benefit to product handling and distribution (Clarke et al., 1988).

The processing conditions of an alginate system for restructured beef steaks was studied by the Meat Industry Research Institute of New Zealand (MIRINZ) in 1994. At 0.6% concentration, the setting time for the highest gel strength (11 N) at 0 $^{\circ}$C was 18-24 hr (Ben and Morris, 1994).

In 1995, MIRINZ conducted the study of four commercial cold-set systems for binding beef pieces: Alginate system, Pearl Meat Binder®, Fibrimex® and Surimi. The alginate systems was used for producing restructured steaks from both comminuted and diced beef. The results indicated that alginate systems provided optimum binding strength, acceptable taste, juicier and more tender than steaks, made from the intact muscle (Esguerra, 1995). Other commercial alginate gel structured meat products already on the market are fish patties and shrimp-like fish products (Imeson, 1992).

4. Protein

Besides hydrocolloid binding, protein binding also plays an important role in fabricated or restructured food products. Protein binding ability is obtained from thermal or heat gelation of functional proteins which are either added or naturally present in the food. Traditionally, milk, meats, eggs, cereals, legumes, and oilseeds have been the major natural sources of food proteins. Protein gelation occurs in many types of

fabricated products: comminuted meat or fish products; vegetable proteins texturized by extrusion or spinning; and reformed meat chunks (Setser, 1992).

Heat-induced protein gelation generally is a two-step mechanism: a dissociation and an aggregation step. In the dissociation step, protein is unfolded or denatured with a temperature change or chemical alteration by reducing the charged groups through pH changes or addition of salt or water-competitive compounds under appropriate conditions. As previously stated, aggregation is the second step in the process and in this step, the protein gel or network is formed and water is entrapped within the three-dimensional network. The protein-water interactions immobilize the water within the gel, and the protein-protein interactions are responsible for forming the gel network. The types of interactions that stabilize the gel differ variably among protein types but can be grouped as the following: hydrogen bonds, disulfide bonds, hydrophobic and electrostatic interactions.

4.1 Egg white albumin

4.1.1 Composition of egg white

Egg consists of approximately 65% albumin and 35% yolk. Egg albumin (white) is a sticky, viscous, heterogeneous material with an approximate volume of 30 ml. Albumin consists of about 88% water, 10.1% protein, 1.23% carbohydrate, and 0.56% ash. Albumin contains as many as 40 proteins, but more than half of these are minor components. Egg albumin proteins can be categorized into two groups: functional

and nonfunctional proteins. Functional proteins are conalbumin, ovomucoid, lysozyme and ovomucin while nonfunctional proteins are ovalbumin and globulins.

4.1.2 Gelation

Egg albumin has the ability to form heat-induced gels. The gelation of egg white protein is the two-step mechanism described earlier as (1) heat-induced denaturation and (2) network formation. In the denaturation step, heat results in the exposure of hydrophobic and sulfhydryl groups. Denatured proteins aggregate to form gel networks via disulfide bonds, and/or hydrophobic interaction (Zabik, 1992).

4.1.3 Application

The gelation of egg white protein is expected to support and bind other ingredients within the gel matrix of food products (Setser, 1992). Egg white has its gel-strengthening ability by composite reinforcing and water-binding capacity (Lee, 1984). Egg albumin is utilized to enhance gel strength in surimi and surimi-based products. The level of frozen raw egg white commonly used in a surimi formulation is 3-10% (weight basis). At the 6% level, surimi gel strength reaches a maximum and beyond 10%, the effect is no longer observed. When raw egg white and starch are added to the protein matrix at the combined level of 10%, the compressive (cohesiveness or gel strength) and penetration forces reached a maximum at 5% starch and 5% egg albumin (Chen et al., 1993).

Also, the gel strengthening of egg white is important in the low temperature setting (0-40 °C) of surimi. Egg white makes the product whiter and glossier, however, a high level of egg white tends to produce an off-flavor in the product.

4.2 Fish Protein

4.2.1 Chemistry of fish protein

The proximate composition of fish is 78-83% moisture, 15-20% protein, 1-4% fat, and 1-1.3% mineral matter. Fish proteins are divided into classes based upon their solubilities. Water-soluble proteins are called albumin and salt-soluble proteins are globulins. Albumin comprises 10-20% of the fish muscle proteins, while 70-90% are globulins.

Structurally, fish muscle contains three fractions of proteins:

(1) sarcoplasmic;

(2) myofibrillar; and

(3) stroma proteins.

Sarcoplasmic proteins, the proteins of the sarcoplasm, comprise about 30% of the total amount of protein and can be extracted by homogenizing the fish muscle with water or neutral salt solutions with ionic strengths below 0.15. Due to the water-soluble ability of the sarcoplasmic protein, the protein is sometimes called simply fish albumin. Fish albumin consists of over 100 various proteins, most of which have enzymic activity.

Myofibrillar proteins are the structural proteins containing myosin, actin, tropomyosin, troponin, and actinin. The proportion of myofibrillar protein in fish meat is about 66-77% of the total protein content. Myofibrillar protein is extracted with neutral salt solutions of ionic strength above 0.15 (Scopes, 1994). Myofibrillar proteins perform a significant role in the coagulation and gel formation of fish meat.

Stroma proteins form the connective tissues and are composed primarily of collagen and elastin. Stroma proteins are insoluble and can not be extracted by water, acid or alkaline solution, and 0.01-0.1 M neutral salt solutions. However, collagen is melted or gelatinized and changed into a water soluble gelatin when fish meat is heated in a moist atmosphere for an extended period of time. On the other hand, elastin is not affected by moist heat.

4.2.2 Application

Sarcoplasmic proteins have been suggested to hinder the gel formation of fish meat by adhering to the myofibrillar protein (Suzuki, 1981). The elasticity of fish meat products is reduced if sarcoplasmic proteins are incorporated within the product or is not removed during washing cycles in the processing steps. Recently, sarcoplasmic proteins extracted from fish were reported to contain an enzyme, (i.e. transglutaminase) that affects fish paste setting. Setting or low temperature gelation is the important step in a heat-gelled fish product and imparts the highest gel strength (Lanier, 1986). The setting time of minced fish varies from species to species. During the initial setting stage, sarcoplasmic proteins increase the breaking force (Nowsad et al.,

1995a) and promote the cross-linking of myosin heavy chain gels (Nowsad et al., 1995b). However, the setting of fish paste still occurs without the addition of sarcoplasmic proteins partly due to non-covalent bonds presumably formed by thermal aggregation.

The ability of sarcoplasmic proteins extracted from beef as a binding agent of meat pieces was previously studied by Macfarlane in 1977. The binding strength of meat pieces made from sarcoplasmic protein exhibited poor cohesiveness when compared with one containing myosin and actomyosin.

5. Transglutaminase(TGase)

5.1 Characteristics of tranglutaminase

The enzyme, tranglutaminase, has the systematic name of protein-glutamine:amine γ-glutamyltransferase, however, it is also known as Factor $XIII_a$ or fibrinoligase. The EC number of transglutaminase is 2.3.2.13.

Transglutaminase is a transferase enzyme which catalyses the acyl transfer reaction. Transglutaminse-catalysed reactions can be categorized into 3 reactions with different acyl acceptors. The acyl donors are the γ-carboxyamide groups of peptide or protein bound glutamyl residues. When transglutaminase uses the primary amine as an acyl acceptor, the acyl-transfer reaction occurs (Fig. 11a). When the ε-amino groups of lysine residues in proteins are acyl acceptor, ε-(γ-Glu)-Lys crosslinks are formed intra- and intermolecularly (Fig. 11b). Without primary amines, water can act as the acyl acceptor and the γ-carboxyamide groups of glutamine residues are deaminated and the

process called deamidation (Fig. 11c). The scheme of transglutaminse-catalysed reactions are shown in Fig. 11 (Anon).

5.2 Sources of Transglutaminase

Transglutaminase has been found in animals, plant tissues, and microorganisms. Since the 1960s, transglutaminase has been only obtained from animal origins, especially guinea pig livers. Due to the scarcity of guinea pig liver as well as the complicated separation and purification processes, other sources have been investigated. In 1989, transglutaminse was found in a culture of *Streptoverticillium sp*. and *Streptomyces sp*. (Nielsen, 1995 and Zhu et al., 1995).

The advantages of using microbial transglutaminase are its lower cost and ease of use. Microbial transglutaminase can be obtained in mass production through microbial fermentation which results in a lower price than commercially transglutaminse purified from guinea pig liver. Also, the microbial transglutaminase is Ca^{2+} independent which is easier to use when compared with tranglutaminase derived from liver which is Ca^{2+} dependent as shown in Table 3 (Anon).

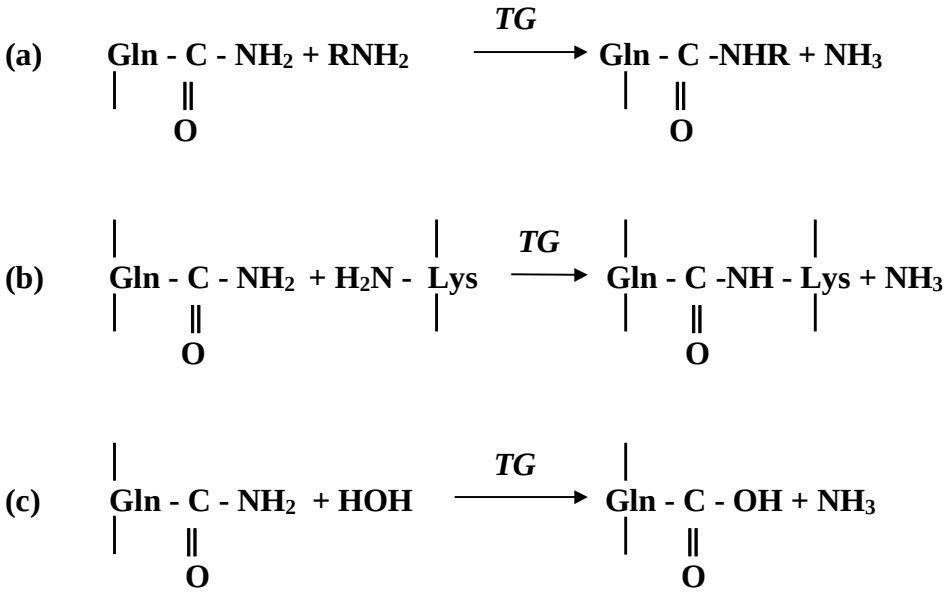

Fig. 11: The reactions catalyzed by transglutaminase (Anon)
(a) acyl-transfer reaction
(b) crosslinking reaction between Gln and Lys residues
(c) deamidation

Table 3: Ca^{2+}-dependency of Microorganism Transglutaminase and Guinea Pig Liver Transglutaminase (Anon)

	Microorganism Transglutaminase	Guinea Pig Liver Transglutaminase
0 mM $CaCl_2$	100%	0%
1 mM $CaCl_2$	100%	39%
5 mM $CaCl_2$	99%	100%

5.3 Measuring Transglutaminase Activity

The activity of transglutaminase is measured by several methods: amine incorporation, disappearance of amino groups, increase in molecular weight, formation of NH_3, measurement of gel strength, measurement of ε-(γ-Glu)-Lys dipeptide, and the amount of incorporated monodansyl cadaverine.

Amine incorporation assays measure the rate of incorporation of [^{14} C] putrecine. The method used for activity determination is the colorimetric hydroxamate analysis described by Folk and Cole (1966).

The disappearance of amino groups or the amounts of remaining amino groups are measured by using the trinitrobenzenesulfonate method. The measurement of the amount of free amino groups is also measured by the fluorescence intensity.

The increase in molecular weight by sodium dodecyl sulfate polyacrylamide gel electrophoreses (SDS-PAGE) principle involves the measurement of myosin heavy chains (MHC) and cross-linked myosin heavy chains (CMHC) or polymer content (>MHC) resulting from the polymerization reaction of transglutaminase. As the reaction progresses, the myosin heavy chains will decrease while the cross-linked myosin heavy chains increase. This method is considered a qualitative rather than quantitative procedure. The detection of NH_3 released assay is based on the conversion of NADPH to $NADP^+$ in the production of glutamate from NH_3 and β-ketoglutarate catalyzed by the glutamate dehydrogenase.

Sakamoto et al. (1994) described the measurement of gel strength from the crosslinking reaction. This method is not time-consuming and allows a high number of

samples to be tested in a short time period. The gel strength was measured in micro titer plates by using a Tensipressor equipped with a 2.5 mm diameter cylindrical plunger.

The measurement of ε-(γ-Glu)-Lys dipeptide (G-L bond) is described by Kamazuwa et al. (1993), while the content of ε-(γ-Glu)-Lys dipeptide was measured by the method of Griffin et al. (1982). The production of ε-(γ-Glu)-Lys dipeptide originated directly from the activity of transglutaminase. Protein from the reaction of transglutaminase is digested with proteinase until only those with G-L bond remain. The sample and the amount of G-L bonds are then analyzed by HPLC. The other method used to measure transglutaminase acitvity is measuring the amount of incorporated monodansyl cadaverine (MDC) as described by Wan et al. (1992).

5.4 Substrate specificity

Ajinomoto U.S.A., Inc. demonstrated the specificity of the microbial transglutaminase enzyme to the type of the substrate (proteins) (Table 4). For meat proteins, gelatin and actin are very good substrates in reacting with the enzyme because of their random structure. The 11S globulin and 7S globulin in soybean, casein and Na-caseinate in milk are also good substrates due to their high lysine content and glutamine residues (Anon). The conformation of the substrate is another major factor affecting the enzyme activity.

Table 4: Reactivity of Microorganism Transglutaminase for Various Proteins (Anon)

Source	Food Protein	Reactivity
Milk	Casein	very well
	Na-caseinate	very well
	α -lactalbumin	depending on conditions
	β-Lactoglobulin	depending on conditions
Eggs	Egg white protein (ovalbumin)	depending on conditions
	Egg yolk Protein	well
Meats	Myoglobin	depending on conditions
	Collagen	well
	Gelatin	very well
	Myofibril: myosin	very well
	Myofibril: actin	mostly does not react
Soybean	11S globulin	very well
	7S globulin	very well
Wheat	Gliadin	well
	Glutenin	well

The alteration of the conformation of 11S seed protein from native to the denatured state improved the activity of the enzyme (Nielsen, 1995).

5.5 Applications

The transglutaminase enzyme has been applied to various protein-based products, such as meat, fish, dairy, and vegetables (Nielsen, 1995). To provide a relatively useful literature review concerning fabricated or restructured food, a focus on meat and fish products will be utilized.

In meat products, Factor $XIII_a$ has been found to crosslink the proteins within the meat itself, such as fibrin and actin, myosin and actin. Transglutaminase also causes the crosslinking between myosin and proteins (soy protein, casein and gluten) commonly used in meat processing which benefits fat-free or low-fat products (Kurth and Rogers, 1984). Transglutaminase causes the gelation of actomyosin from beef and mechanically deboned poultry. Transglutaminase can be used in combination with fibrinogen and thrombin in producing restructured meat from pieces of meat at a temperature below 10 $^{\circ}C$ with an overnight reaction time (Paardekooper, 1987). Sakamoto (1994) stated that meat pieces could be bonded together by microbial transglutaminase without heating, and with the addition of salt and phosphates to produce a restructured fresh meat product. The utilization of transglutaminase enables by-products (mechanically deboned meat, collagen) to be used for the production of restructured foods.

In restructured fish products, such as surimi (*Alaska pollock*), Lanier (1986) stated that gelation at low temperatures or setting is of importance to achieve the highest gel strength which occurs at 25 °C. The effect of endogenous transglutaminse extracted from fish species on the setting of various actomyosin paste (Alaska pollock, sardine, common horse mackerel, flounder and Spanish mackerel) was studied by Nowsad et al. (1995). The result was that transglutaminse promoted the cross-linking of myosin heavy chains during its setting. Actomyosin gel strength measured from breaking force increased with the addition of extracted transglutaminase.

The effect of added microbial transglutaminase at a low temperature setting of Alaska pollock was also studied by Lee et al. (1997). The result obtained from this study supported the previous study. With an increase in setting time and constant quantity of microbial transglutaminase (MTGase), gel strength is improved. The higher MTGase, the greater the gel strength. Also, gel strength correlated with increasing amounts of nondisulfide polymerization and ε-(γ-Glu)-Lys dipeptide content.

Objectives in this study are to produce restructured seafoods from processing by-products and underutilized species by using heat-set and cold-set binders based on their functional properties especially gel formation property.

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Section II: Effect of starch, egg white albumin, and fish sarcoplasmic protein on the textural and cooking properties of restructured squid (Illex illecebrosus)

Abstract

Restructured squid was produced from the underutilized species of North Atlantic short-finned squid (*Illex illecebrosus*) with selected heat-set binders (starch, egg white albumin, and fish sarcoplasmic protein). The effects of type and level of each heat-set binder, as well as their combinations, were evaluated for their textural and cooking properties. The levels of each heat-set binder ranged from 2, 4, 6, 8, to 10%. When the level of starch was increased from 2 to 10%, a decrease of hardness, cohesiveness, and springiness was observed. However, 2% egg white albumin increased the hardness and cohesiveness while 2% fish sarcoplasmic protein improved cohesiveness and springiness. When starch was combined with either egg white albumin or fish sarcoplasmic protein to the combined level of 10%, all textural parameters decreased.

In general, starch had no ability to improve cohesiveness as an index of gel strength when combined with egg white albumin and fish sarcoplasmic protein. However, starch-based combinations are able to lower the cooking loss of restructured squid when compared to those produced from starch, egg white albumin, and fish sarcoplasmic protein separately.

Introduction

Squid or calamari is a common seafood consumption item in Europe and Asia. The Japanese have eaten squid as a traditional food for centuries with an annual consumption exceeding 2 kg per capita, which is more than any other marine species (Ke et al., 1991). Squid, however, has low consumer acceptability in the United States due to its tough rubbery texture and most of the consumption centers around certain ethnic groups (Otwell and Hamann, 1979).

In recent years, squid usage in the United States and Canada has dramatically increased. The annual consumption has risen about 15-20 percent as the general population is becoming more acquainted with the product. The domestic squid market, once scarce, is now experiencing a rapid increase. United States processors are now seeking new suppliers for imported cleaned squid to satisfy their markets (Ring, 1995).

Two important Atlantic squid species harvested along the Atlantic coast of North America from the northwest to the West-central coast are the Atlantic short-fin squid (*Illex illecebrosus)* and Atlantic long-fin squid (*Loligo pealei)* which is considered the gourmet squid of choice for the marketplace. With increasing domestic demands, nearly 50% of the *Loligo pealei* catch on the East Coast is now used for consumption in the United States and Canada. *Illex illecebrosus* has a smaller and thinner mantle than *Loligo pealei,* a more leathery texture, and is primarily used as bait. The recognized difference in texture significantly influences market acceptability. The resource status of *Illex illecebrosus* is considered as under exploited. The under exploitation of squid results from various reasons, such as the lack of handling and processing equipment, fluctuating

stocks, and susceptibility of squid to spoilage and quality loss (Stanley and Hultin, 1982). This study was encouraged by personnel from the L.D. Amory Seafood Company in order to make an underutilized resource a more profitable fishery.

The general definition of engineered or restructured foods are those foods prepared from individual ingredients or components to produce finished products having specified properties and appealing forms. Fabrication of seafood products usually starts with comminuting raw materials, combining matrix binding agents, and then shaping the product with a mechanical extruder. The suitable binding-matrix agent at a proper level should retain the desired shape when the mixture is passed through an extruder (Soo and Sander, 1977a).

Since an extruder specifically used for the production of restructured squid rings is not readily available, restructured squid patties were fabricated and used for measuring textural parameters in this study. Fabricated patties were first used to predict the sensory response to textural parameters of seafood products in 1977. In that study, fabricated cooked shrimp patties were used as representatives of extruded, breaded shrimp shapes. The textural parameters of fabricated cooked shrimp patties correlated well with the sensory response of the shrimp shapes. The advantage of the patties is the elimination of expensive and time consuming pilot plant operations to produce a sufficient quantity of shrimp shapes required to measure single formula and process parameters (Soo and Sander, 1977b).

The most common system utilized for fabricating comminuted raw materials are heat-set binders. The basic mechanism of a heat-set binder system involves the

incorporation of salt and phosphates to solubilize the myofibrillar proteins (myosin) in muscle tissue during the comminuting or mincing stage. Upon heating, the extracted myofibrillar proteins will gel by protein-protein interactions resulting in the binding of comminuted meat (Schmidt et al., 1981).

The objectives of this study were: (1) To develop processing steps for restructured squid production from Atlantic short-finned squid (*Illex illecebrosus*) and (2) To evaluate the effects of type and level of each heat-set binding matrix agent or binder as well as their combinations have on the textural and cooking properties of restructured squid. The two major categories of heat-set binders, proteins and hydrocolloids, were investigated. The effects of fish sarcoplasmic protein, egg white albumin and modified corn starch used independently on the textural characteristics of an restructured squid product were evaluated using objective analyses. Also, the combination effects between starch and egg white, and starch and fish sarcoplasmic protein were investigated. The Texture Profile Analysis (TPA) parameters: hardness; cohesiveness; and springiness were used to determine the effects of each binding material.

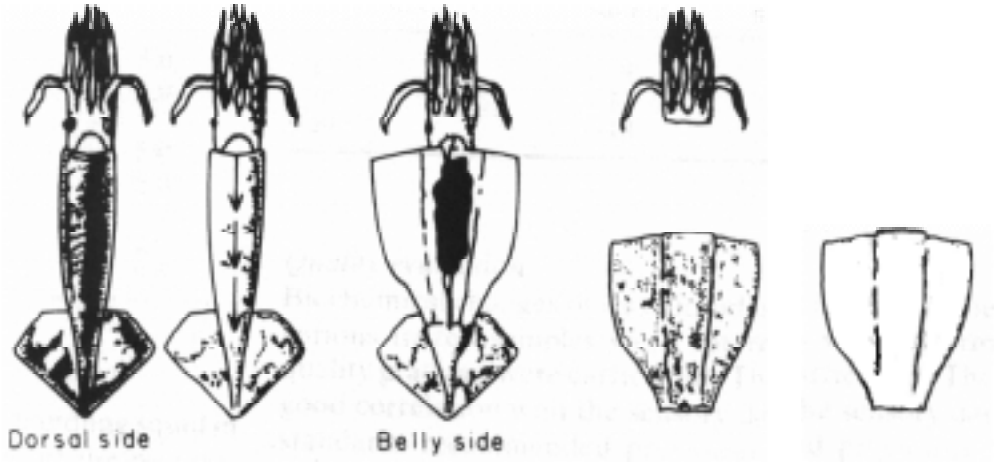
Materials and Methods

Materials

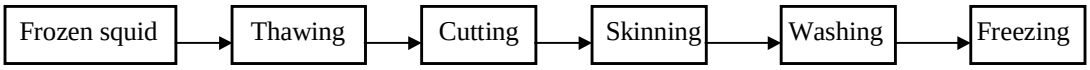
Frozen Atlantic short-finned squid (*Illex illecebrosus)* supplied from the L.D. Amory Seafood Co., in Hampton, VA was used throughout the investigation. Modified corn starch (Mira-Gel ® 463) was provided by the A.E. Staley Manufacturing Company (Decatur, IL). Fish albumin was extracted from frozen Atlantic croaker (*Micropogan undulatus*) caught in the Chesapeake Bay and frozen within 24 hr after harvest. The fish were maintained in the frozen state (-20 °C) until extraction. Egg white was separated from fresh chicken eggs purchased from the Kroger Company.

Squid meat preparation

According to Ke et al. (1991), the most suitable method for thawing Atlantic short-fin squid is at 5-10 °C for 20-30 hr in air or at 6-12 °C for 8-14 hr in seawater. The thawing method used in this study followed the method recommended by Ke et al. (1991). Blocks of frozen squid (33 lb.) were air thawed at 5-10 °C for 24 hr. The squid tubes or mantles were cut along the belly side to remove the viscera and pens (cartilaginous backbone). The heads, tentacles, and fins were subsequently removed as illustrated in Fig. 12. The squid mantles were subsequently hand-skinned to remove both the skin and the pigment cells and then washed in the chilled water (5 °C). The milky



(a)



(b)

Fig. 12: (a) Details of procedure on cutting and skinning operations
(b) The flow chart of squid meat preparation (Adapted from Ke et al., 1991)

white cleaned squid mantles were placed in polyethylene freezer bags and frozen at –20 °C in a walk-in blast freezer until used in the study.

Fish sarcoplasmic protein preparation

The extraction method for fish sarcoplasmic protein isolation followed the preparation of extracts from animal tissues by Doonan (1996). The fish species selected for this study was Atlantic croaker (*Micropogan undulatus*) due to its high content of white meat and high gelling property. Atlantic croaker was gutted, filleted, skinned, and cleaned in chilled water. The cleaned fillets were trimmed of visible fat. The fish muscle was then cut into the pieces weighing approximately 5 g and placed in a pre-cooled Waring blender (Waring Commercial, New Hartford, CT) with 2 vol. of cold water (5 °C). The fish tissues were disintegrated or homogenized 4 times for 30 sec each at medium speed. The homogenate was poured into a glass beaker, placed in an ice bath maintained at 0-2 °C, and stirred for 15-30 min to ensure maximum extraction of the soluble protein. Subsequently, the homogenate was centrifuged at 4 °C in a refrigerated centrifuge (Sorvall RC2-B, Ivan Sorvall Inc., Norwalk, CT) at a speed of 6500 rpm (5000×g) for 30 min. The supernatant was carefully removed from the centrifuge bottles without disturbing the pellet or sediment matter. To concentrate the protein extract, the supernatant was dialyzed against 20% (w/v) polyethylene glycol (MW 20,000+) in a cellulose membrane dialysis tubing that retains molecular weight over 12,000 (Sigma Chemical Co., St. Louis, MO) for 24 hr with constant stirring. Polyethylene glycol, high

molecular weight substance, was used to remove water from the dialysis bag without penetration of the protein through the dialysis membrane (Scopes, 1994).

Restructured squid patty preparation

Restructured squid patties were prepared by mincing tempered squid meat with 0.5% salt for 3 min in a food processor (Cuisinart Model DLC-8M, Cuisinart Corp., East Windsor, NJ). Then matrix-binding agent with 0.5% sodium tripolyphosphate (STP) was added to the squid pastes. The mixture was thoroughly blended for an additional one minute at medium speed. Blended material from each treatment was formed into 80 g patties using 100×15 mm disposable polyethylene Petri dishes (Fisher Scientific Co., Suwanee, GA) coated with lecithin. The formed patties were placed in polyethylene bags and immediately frozen at –20 $^{\circ}$C in walk-in blast freezer. The flowchart for preparation of the restructured squid patties is shown in Fig. 13.

Addition of starch, egg white albumin, and fish sarcoplamic protein

To investigate the effect of each binding material: modified corn starch (Mira-Gel® 463); raw egg white albumin; and fish sarcoplasmic protein, each binding material was added separately to the squid pastes (after mincing stage) at 0, 2, 4, 6, 8, and 10%

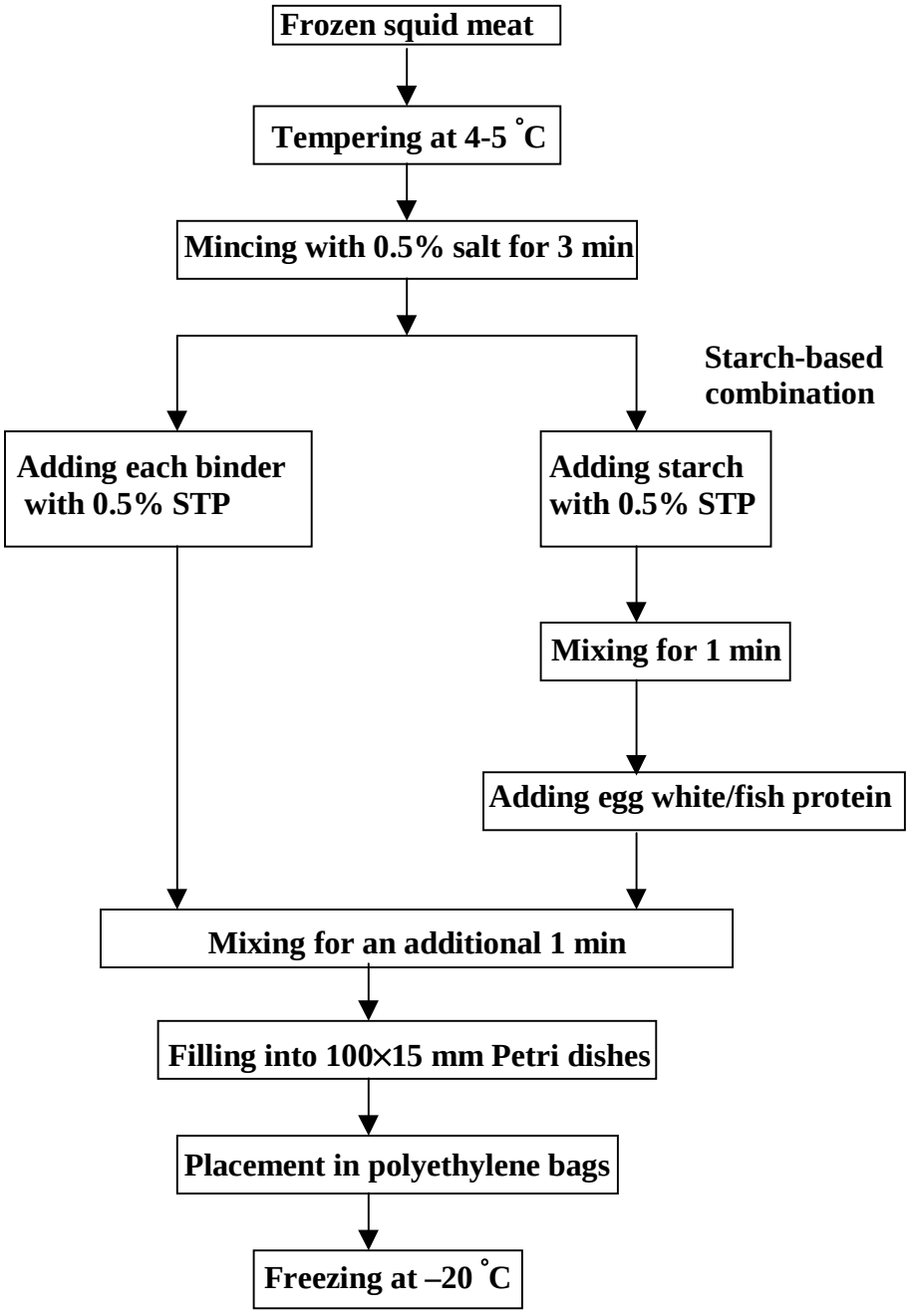


Fig. 13: The flowchart of restructured squid patty preparation

level with 0.5% STP. The mixtures were then mixed at a medium speed for an additional 1 min as shown in Fig. 13.

To investigate the starch-based combination effect (starch and egg white, starch and fish sarcoplasmic protein), starch at the level of 0, 2, 4, 5, 6, 8, and 10% and 0.5% STP were added to the squid pastes after the mincing stage. The mixture was blended for one minute to evenly incorporate starch into the mixture. Egg white/fish sarcoplasmic protein at the decreasing level of 10, 8, 6, 5, 4, 2, and 0% was added to the blended mixture for a total combined level of 10%. The mixture was again blended for an additional 1 min as illustrated in Fig. 13. In the starch-based combinations, starch was first added to the mixture as shown in the flowchart (Fig. 13) to prevent immediate gelling of the mixture.

Cooking procedures

Frozen restructured squid patties were removed from the freezer and cooked in the frozen state by deep fat frying in vegetable oil at 150 $^{\circ}$C for 8-10 min. After cooking, the squid patties were cooled to room temperature and blotted dry with paper towels for Instron measurements.

Cooking loss

The weights of the restructured squid patties were measured before and after cooking.

Cooking loss (%) = raw wt. - cooked wt. / raw wt. * 100

Instron measurement of Texture Profile Analysis (TPA) parameters

The procedure used for instrumental Texture Profile Analysis was similar to that described by Bourne (1982a and 1982b). Two frozen restructured squid patties from each treatment were cooked and cooled to room temperature as previously described, and measured for their TPA parameters with an Instron Universal Testing Machine (Model 1011, Instron Corp., Canton, Mass.). Three three-quarter-inch diameter cores were removed from each cooked patty and the samples height trimmed to 1 cm. Samples were compressed to 70% of their original height using the 14.5 cm flat compression head attached to the Instron crosshead. A 50 kg load cell was used with 40% of the load range at the crosshead and a chart speed of 100 mm/min. Two compression cycles per slices were generated to form a "two-bite" work-force compression curve.

Textural parameters derived from two successive compressions were hardness, cohesiveness, and springiness. Deformation curves were plotted and averaged for hardness (peak force of the first compression, kg), cohesiveness (total energy of 2^{nd} compression ÷ total energy of 1^{st} compression × 100), and springiness (base width of 2^{nd}

compression ÷ base width of 1st compression × 100) for each sample. The calculation for springiness followed Claus (1995).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) with the Statistical Analysis System (SAS 6.12) to evaluate significant differences among treatments. Duncan's Multiple Range test was used to determine differences between treatment means when analysis of variance indicated a significant difference at the $P \leq 0.05$ level. All measurements were derived from 6 samples per treatment/replication.

Results and Discussions

Effect of starch

Differences ($P<0.05$) between restructured squid for instrumental hardness, cohesiveness, and springiness values are reported in Table 5. Increasing the level of starch from 2 to 10% significantly affected the textural properties of the restructured squid with a similar pattern for all parameters (Fig. 14) i.e., a decrease of hardness, cohesiveness, and springiness resulted. At the 2% level, all textural measurements reached the maximum values (hardness = 6.3 Kgf; cohesiveness = 40.5%; and springiness = 77.4%), however, the cohesiveness and springiness were not significantly different ($P<0.05$) from the control (no added starch). These results suggested that the addition of starch did not improve the cohesiveness and springiness of the deep fat fried restructured squid.

Normally, the addition of starch in the comminuted protein gel matrix (e.g. surimi) increases the gel strength and the cohesiveness due to its ability to swell, absorb, and bind the surrounding water in the gel when gelatinized. Consequently, the gel become more compact and firm in the confined casing (Kim and Lee, 1987; Lee et al., 1992; and Chen et al., 1993).

In a gel produced from giant squid (*Dosidicus gigas)*, the result of gel-enhancing ingredients such as hydrocolloids (e.g. starch and carrageenan) was totally different from that found in surimi. Hydrocolloids have been reported as incapable of improving poor

gel-forming capacity in giant squid. Addition of carrageenan (2%) and starch (5%) produced a significant decrease in gel strength with respect to the control at either the 1.5% or 2.5% salt level (Gomez-Guillen and Montero, 1997).

Effect of egg white albumin

The effect of egg white albumin on hardness and cohesiveness was significantly different ($P<0.05$) from the control but not significantly different ($P>0.05$) for springiness (Table 6). Added egg white albumin from 2 to 10% slightly improved the hardness and cohesiveness of deep fat fried restructured squid (Fig. 15). At the 2% egg white level, restructured squid showed maximum hardness (9.1 Kgf) and increased cohesiveness when compared to the control ($P<0.05$). The cohesiveness was not significantly different from each other ($P>0.05$) within the range of 2, 4, 6, 8, and 10%. Therefore, no specific level of egg white yielded maximum cohesiveness.

In general, 2% added egg white albumin improved the hardness and cohesiveness of the deep-fat fried restructured squid.

The role of egg white on the textural properties of protein gel has not been extensively described. The effect of egg white on the hardness of a comminuted protein gel was studied by Montero and Gomez-Guillen (1996) respectively. They found that added egg white had a direct positive effect on the hardness of sardine surimi (*Sardina pilchardus).*

The gel-strengthening effect of egg white in surimi gel and giant squid gel was studied by Chen et al. (1993) and Gomez-Guillen and Montero (1997). The highest

surimi gel strength in accordance with the minimum expressible moisture was observed at 6% raw egg white. Iso et al. (1985) suggested that egg white absorbed water within the network structure without increasing of the number of protein crosslinks in the gel. The other possible reason for gel-strengthening effect of egg white in surimi came from its protease inhibitory property (An, 1997). Protein additives (e.g. egg white, beef plasma protein, potato powder, and whey protein concentrate) has been used to inhibit the proteolytic activity and enhance the gel strength in Pacific Whiting and Arrowtooth Flounder surimi. (Wasson et al., 1992; Reppond and Babbitt, 1993; and Morrissey et al., 1993).

The result of adding egg white in surimi gel differed from that in giant squid gel (*Dosidicus gigas*) in that addition of 2% non-muscle proteins (e.g. egg white, soy protein, and gluten) in giant squid gel did not improve the gel strength (Gomez-Guillen and Montero, 1997). Their study indicated different results from our research in that our study found improvement in cohesiveness in restructured squid (*Illex illecebrosus*) with added egg white (2 to 10%) compared to the control, the difference may have resulted from the species of the squid used.

Effect of fish sarcoplasmic protein

The result of added fish sarcoplasmic protein is shown in Fig. 16. Differences ($P<0.05$) in restructured squid with the addition of fish protein on hardness, cohesiveness, and springiness values are contained in Table 7. The cohesiveness and springiness of restructured squid with 2, 4, 8 and 10 % of fish albumin were significantly different from the control ($P<0.05$). Among the levels of added fish albumin from 2 to 10%, no differences ($P<0.05$) was found on cohesiveness and springiness. Hence, no specific level of fish albumin yielded the maximum cohesiveness and springiness. In addition to the cohesiveness and springiness, fish proteins at 4, 8, and 10% also affected the hardness, as the hardness values were higher than the control ($P< 0.05$). When these results were compared to those obtained from the addition of egg white and starch described earlier, fish sarcoplasmic protein had an ability to improve cohesiveness as that found in egg white but not in starch.

Sarcoplasmic protein in fish muscle is usually removed during the washing process in the production of surimi due to its ability to weaken the gel. Recently, sarcoplasmic protein was determined to enhance the gel strength to some extent and this property may be due to an endogenous enzyme (Nowsad et al., 1995). Only limited research has been performed to study the effect of sarcoplasmic protein on the gelling ability. From our results, however, sarcoplasmic protein had the ability to enhance the gel strength of restructured squid.

Effect of starch and egg white combination

The effect of starch and egg white on the textural parameters when used separately was previously described. Starch had a negative effect on the hardness, cohesiveness, and springiness of the gel. As the level of starch increased, all parameter values decreased. Egg white albumin improved the cohesiveness of the gel and enhanced the gel hardness when compared to the control.

To study the combination effect between starch and egg white that may have occurred, starch and egg white were combined at various ratios to a level of 10%. Starch ranged from 0, 2, 4, 5, 6, 8, to 10% while egg white proportionately decreased from 10, 8, 6, 5, 4, 2, to 0%. Differences ($P<0.05$) were found between restructured squid for hardness cohesiveness, and springiness values (Table 8). The combination between starch and egg white did not improve the cohesiveness and springiness since the combined level (0:10) yielded the highest cohesiveness and springiness was found not significantly different ($P>0.05$) from the control. At the 0:10 (starch: egg white) level, the hardness was greater than the control ($P<0.05$). However, this level can not be a usage level due to the absence of starch in the combination i.e., the result was produced only from the 10% egg white which was reported earlier.

In general, all textural parameter values decreased as the starch level increased (Fig. 17). The effect followed the same pattern when starch was used alone as described in the previous section, indicating that egg white had no synergistic effect with starch.

The starch and egg white combination effect on the gel strength of giant squid was also similar to that obtained in this study in that the combination decreased the gel strength (Gomez-Guillen and Montero, 1997).

Effect of starch and fish sarcoplasmic protein combination

The study of the combination effect between starch and fish albumin was conducted in the same manner as the starch and egg white combination as described above. The combination level was set for the total of 10% by increasing starch from 0, 2, 4, 5, 6, 8 to 10% and decreasing fish protein from 10, 8, 6, 5, 4, 2, and 0% respectively.

Differences ($P<0.05$) between restructured squid for hardness cohesiveness, and springiness values were observed (Table 9). At 0:10 (starch: fish protein) level, the highest hardness (8.6 Kgf) was achieved. The 0:10 combination level yielded the highest springiness (85.7%) value, but it was not significantly different ($P>0.05$) from the control. This combination level (0:10) also yielded the highest degree of cohesiveness and was statistically different ($P>0.05$) from the control, however, this usage level can not be considered due to the absence of starch. That is, the results are produced only from 10% fish protein which was reported earlier.

In general all textural parameter values decreased as a function of starch concentration (Fig. 18). Again, when the results were compared with those obtained from starch and fish protein used separately, the results were similar to that found when starch was used as the only ingredient. From the data obtained, it can be concluded that

starch had the predominant effect over fish albumin due to the reduction in gel hardness, cohesiveness, and springiness. Since no combination levels showed an improvement in the cohesiveness values which was the primary focus of this study, no combination levels can be recommended.

The cooking loss

Cooking loss from deep-fat frying the various restructured squid formed with each type of binder (starch, egg white albumin, and fish sarcoplasmic protein) and the combination of binders (starch and egg white, starch and fish sarcoplasmic protein) are presented in Table 10. Restructured squid produced with 4, 6, 8 and 10% starch had the lower cooking losses than the control ($P<0.05$) while the 2% starch level was not statistically different from the control ($P>0.05$). Cooking loss was reduced when the level of added starch increased (Fig. 19). This can be explained by the gelatinization of starch which occurs during the heating process. Gelatinized starch is capable of absorbing water present in the protein matrix within its network structure. The higher the amount of starch contained within the protein matrix, the greater amount of water that can be absorbed. Apparently, the absorbed water also had a negative effect on the reduction of hardness, cohesiveness, and springiness of the gel as described earlier.

The effect of egg white and sarcoplasmic fish protein on cooking loss was less than that found in starch (Figs. 20 and 21). Egg white albumin and fish protein have the capacity of holding water within its structure due to the difference of some treatments

when compared to the control. The egg white levels that had the lower cooking loss ($P<0.05$) than the control were 4, 6, and 8%. Fish sarcoplasmic protein having ($P<0.05$) lower cooking losses than the control were 4 and 6%. The 10% egg white and 8, 10% fish protein did not present the lowest cooking loss as anticipated. This observation may be due to the gel matrix disruption as reported in surimi gel when 10% egg white was added (Chen et al., 1993).

The cooking losses of restructured squid prepared with starch-based combinations were also studied. Starch was combined with egg white/fish protein and the results were similar to that previously obtained when starch was used alone (Fig. 22 and 23, Table 11), i.e., the cooking losses in both combinations decreased as a function of starch content. Starch and egg white combinations having significantly ($P<0.05$) lower cooking loss than control were 2:8, 4:6, 5:5, 6:4, 8:2 and 10:0. At the 0:10 (starch: egg white) level, the cooking loss was higher than the control (no starch and no egg white) because of the disruption effect of egg white as described previously. In the starch and fish albumin combinations, the levels that had significantly ($P<0.05$) lower cooking loss than the control were 4:6, 5:5, 6:4, 8:2 and 10:0. The first two combination levels, 0:10 and 2:8, yielded higher cooking losses than the control again due to the disruption effect of fish protein at 8 and 10%.

Conclusions

Various heat-set binders and their combination effects on the textural parameters and the cooking loss of deep-fat fried restructured squid were studied. Starch had the most evident effect among the binders on the decrease in the hardness, cohesiveness, and spinginess. 2% egg white albumin improved the cohesiveness and hardness of restructured squid while 2% fish sarcoplasmic proteins improved cohesiveness and springiness. When starch was combined either with egg white albumin or fish sarcoplasmic protein (starch-based combination), hardness, cohesiveness, and springiness decrease as a function of starch content presented in the protein matrix.

Despite the fact that starch-based restructured squid contained the weaker gel strength than those formulated with only starch, egg white, and fish protein, the cooking losses of starch-based restructured squid were lower than the control. The cooking loss is the factor that should be taken into consideration for the production of restructured squid rather than focusing on the gel strength alone.

The recommendation for restructured squid production is the use of starch and egg white at 2:8 percent to yield a high percent (43.8) cohesiveness and a more homogeneous product than those of the other starch and fish sarcoplasmic protein combinations and starch, egg white albumin, and fish sarcoplasmic protein alone.

Table 5: The effect of 2, 4, 6, 8, and 10% starch on the hardness, cohesiveness, and springiness of deep-fat fried restructured squid

Level of starch	Hardness (Kg f)	Cohesiveness (%)	Springiness (%)
0- control	4.9 ± 1.54^{b}	39.5 ± 4.46^{a}	76.5 ± 5.36^{a}
2	6.3 ± 1.03^{a}	40.5 ± 4.90^{a}	77.4 ± 4.96^{a}
4	3.7 ± 0.52^{c}	31.8 ± 3.69^{b}	67.1 ± 5.65^{b}
6	3.1 ± 0.43^{d}	28.9 ± 4.24^{b}	62.1 ± 6.69^{c}
8	2.4 ± 0.33^{e}	24.1 ± 5.28^{c}	53.4 ± 9.46^{d}
10	2.3 ± 0.34^{e}	19.8 ± 5.16^{d}	45.5 ± 10.75^{e}

[abcde] Means in the same column followed with same letter are not significantly different ($P > 0.05$) using One-way Analysis of Variance (ANOVA) with Duncan's new multiple range test procedure.

Means for each treatment were calculated from n = 18 from three replications (n = 6 /replication)

Table 6: The effect of 2, 4, 6, 8, and 10% egg white on the hardness, cohesiveness, and springiness of deep-fat fried restructured squid

Level of egg white	Hardness (Kgf)	Cohesiveness (%)	Springiness (%)
0- control	4.9 ± 1.54^{c}	39.5 ± 4.46^{b}	76.5 ± 5.36^{a}
2	9.1 ± 1.94^{a}	45.7 ± 3.02^{a}	79.3 ± 4.48^{a}
4	7.2 ± 1.28^{b}	45.9 ± 4.46^{a}	79.8 ± 6.58^{a}
6	7.3 ± 2.06^{b}	45.5 ± 3.69^{a}	79.0 ± 5.60^{a}
8	6.7 ± 1.09^{b}	43.7 ± 2.99^{a}	75.9 ± 5.27^{a}
10	7.9 ± 1.62^{b}	45.5 ± 4.03^{a}	78.5 ± 6.30^{a}

[abc] Means in the same column followed with same letter are not significantly different ($P > 0.05$) using One-way Analysis of Variance (ANOVA) with Duncan's new multiple range test procedure.

Means for each treatment were calculated from n = 18 from three replications (n = 6 /replication)

Table 7: The effect of 2, 4, 6, 8, and 10% fish albumin on the hardness, cohesiveness, and springiness of deep-fat fried restructured squid

Level of fish albumin	Hardness (Kgf)	Cohesiveness (%)	Springiness (%)
0- control	4.9 ± 1.54^{b}	39.5 ± 4.46^{b}	76.5 ± 5.36^{b}
2	6.0 ± 1.97^{ab}	44.5 ± 6.21^{a}	78.9 ± 7.73^{a}
4	6.3 ± 1.24^{a}	47.1 ± 5.90^{a}	81.9 ± 6.57^{a}
6	4.9 ± 1.33^{b}	43.1 ± 7.22^{ab}	76.5 ± 10.95^{ab}
8	6.5 ± 2.09^{a}	45.4 ± 6.79^{a}	80.7 ± 8.77^{a}
10	6.5 ± 1.86^{a}	46.2 ± 4.58^{a}	80.5 ± 4.92^{a}

[ab] Means in the same column followed with same letter are not significantly different ($P > 0.05$) using One-way Analysis of Variance (ANOVA) with Duncan's new multiple range test procedure.

Means for each treatment were calculated from n = 18 from three replications (n = 6 /replication)

Table 8: The effect of starch:egg white (%) at 0:10, 2:8, 4:6, 5:5, 6:4, 8:2, and 10:0 level on hardness, cohesiveness, and springiness of deep-fat fried restructured squid

Level of starch: egg white	Hardness (Kgf)	Cohesiveness (%)	Springiness (%)
Control	5.2 ± 1.06^{b}	43.1 ± 1.91^{a}	80.2 ± 2.62^{ab}
0:10	6.6 ± 1.44^{a}	45.3 ± 4.84^{a}	82.8 ± 5.88^{a}
2:8	5.9 ± 0.37^{ab}	43.8 ± 2.88^{a}	82.0 ± 4.29^{a}
4:6	4.3 ± 0.53^{c}	35.9 ± 5.71^{b}	73.3 ± 8.26^{bc}
5:5	4.0 ± 0.36^{c}	31.7 ± 2.08^{bc}	68.2 ± 2.87^{c}
6:4	2.8 ± 0.26^{d}	30.4 ± 4.45^{c}	66.2 ± 5.80^{c}
8:2	2.8 ± 0.37^{d}	24.2 ± 3.89^{d}	57.0 ± 6.69^{d}
10:0	2.1 ± 0.31^{d}	19.0 ± 4.36^{e}	44.6 ± 11.33^{e}

[abcde] Means in the same column followed with same letter are not significantly different ($P > 0.05$) using One-way Analysis of Variance (ANOVA) with Duncan's new multiple range test procedure.

Means for each treatment were calculated from n = 18 from three replications (n = 6 /replication)

Table 9: The effect of starch:fish sarcoplasmic (%) at 0:10, 2:8, 4:6, 5:5, 6:4, 8:2, and 10:0 level on hardness, cohesiveness, and springiness of deep-fat fried restructured squid

Level of starch: fish albumin	Hardness (Kgf)	Cohesiveness (%)	Springiness (%)
Control	5.2 ± 1.06^{c}	43.1 ± 1.91^{b}	80.2 ± 2.62^{ab}
0:10	8.6 ± 1.16^{a}	51.0 ± 1.72^{a}	85.7 ± 0.01^{a}
2:8	6.9 ± 1.32^{b}	46.0 ± 5.57^{b}	83.8 ± 4.52^{a}
4:6	3.6 ± 0.38^{de}	37.0 ± 2.71^{c}	76.2 ± 3.23^{b}
5:5	3.8 ± 0.50^{d}	34.8 ± 3.45^{cd}	74.0 ± 3.04^{bc}
6:4	2.7 ± 0.39^{ef}	31.0 ± 2.57^{de}	69.8 ± 2.75^{c}
8:2	2.3 ± 0.29^{f}	27.7 ± 2.82^{e}	63.3 ± 6.54^{d}
10:0	2.1 ± 0.31^{f}	19.0 ± 4.36^{f}	43.8 ± 10.66^{e}

[abcdef] Means in the same column followed with same letter are not significantly different ($P > 0.05$) using One-way Analysis of Variance (ANOVA) with Duncan's new multiple range test procedure.

Means for each treatment were calculated from n = 18 from three replications (n = 6 /replication)

Table 10: The cooking losses of deep-fat fried restructured squid with starch, egg white albumin, and fish sarcoplasmic protein.

Binders	Cooking loss (%)					
	control	2%	4%	6%	8%	10%
starch	54.1 [a]	50.2 [a]	35.0 [b]	28.3 [c]	21.9 [d]	19.1 [d]
	(2.66)	(3.70)	(3.70)	(0.12)	(0.49)	(2.35)
egg white albumin	54.1 [a]	56.0 [a]	49.5 [b]	50.3 [b]	49.6 [b]	55.2 [a]
	(2.66)	(0.95)	(0.73)	(1.32)	(4.10)	(1.95)
fish sarcoplasmic protein	54.1 [ab]	54.5 [ab]	52.6 [b]	52.0 [b]	56.1 [ab]	59.6 [a]
	(2.66)	(0.69)	(4.87)	(1.41)	(1.75)	(2.90)

[abcd] Means in the same row with same superscript were not significantly different from each other ($P > 0.05$) using ANOVA with Duncan's new multiple range test procedure

The numbers in the parenthesis under the means were the standard deviations.

Table 11: The cooking losses of deep-fat fried restructured squid with the combination of starch and egg white albumin and starch and fish sarcoplasmic protein.

Binder	Cooking loss (%)							
	control	0:10	2:8	4:6	5:5	6:4	8:2	10:0
starch : egg white	46.3[b] (1.21)	54.2[a] (0.37)	44.2[c] (0.02)	37.9[d] (1.46)	36.1[d] (0.90)	20.6[e] (0.85)	16.4[f] (0.49)	17.5[f] (0.95)
starch : fish albumin	46.3[b] (1.21)	57.1[a] (0.39)	53.0[a] (1.22)	36.8[c] (0.80)	35.2[c] (4.38)	26.7[d] (0.39)	20.5[e] (3.11)	17.5[e] (0.95)

[abcdef] Means in the same row with same superscript were not significantly different from each other (P>0.05) using ANOVA with Duncan's new multiple range test procedure

The numbers in the parenthesis under the means were the standard deviations.

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<u>Section III</u>: The effect of cold-set binders: alginates and transglutaminase on the physical properties of restructured scallops

Abstracts

Restructured scallops (*Argopecten gibbys)* were prepared with two cold-set binders: alginate and microbial transglutaminase at the 1% level. The different setting times ranged from 2, 6, 9, 12, 18, and 24 hr at 5 °C. A physical property evaluation of binding strengths was performed with compression tests on the MTS Universal Testing Machine. Apparent modulus of elasticity, 5% secant modulus, 10% secant modulus, and 20% secant modulus were used to evaluate the effect of setting times on the binding strengths of restructured scallops. No significant differences ($P>0.05$) in the binding strengths as measured by 5, 10, and 20% secant modulus were obtained. Apparent modulus of elasticity revealed the significant differences in the binding strengths ($P<0.05$). Restructured scallops with alginate gel achieved the highest binding strength (158.7 kPa) at 2 hr setting, while restructured scallops with microbial transglutaminase obtained the highest binding (336.0 kPa) at 24 hr setting.

Introduction

Traditionally, restructured meat production requires either heat or a hot-set binder process to bind meat proteins, non-meat proteins (such as milk or soybean protein), and hydrocolloids (such as alginate and starches) in a system. Therefore, these products have to be sold frozen and cooked from the frozen state. With the demand for raw and chilled restructured meats, a market for cold-set binders was developed (Ben and Morris, 1994).

Cold-set binders enable food processors to utilize the processing wastes (such as trimmings, cuts, or small pieces of meat) to make more desirable and more acceptable products that can be sold as raw and chilled (Esguerra, 1995). Cold-set binding systems developed for restructured meats are alginate systems, Pearl Meat Binders, Fibrimex, and surimi. Pearl Meat Binders (a Japanese product) at 1-2% of meat weight produced a strong bind, an acceptable taste and aroma, however, a time-consuming process to coat each piece with the powder is required. Fibrimex, the Netherlands product containing fibrinogen and thrombin, is used to bind meat pieces by the reaction of fibrinogen and thrombin. Fibrimex has many advantages: good binding strength; a fast setting time (10-15 min); and a controllable gel strength (by adjusting the concentration of fibrinogen). However, the product labeling must list the word “blood” or “plasma” on the ingredients label thereby imparting an unfavorable connotation to consumers (Esguerra, 1995).

Surimi has been suggested as a potential cold binder in the production of restructured meats due to its ability to form a gel at low temperatures (40 $^{\circ}$C) (Esguerra, 1995). The use of surimi as a cold-set binder, however, has not been extensively studied.

Most of the research performed on surimi focused on its application as a hot-set binder in restructured beef steaks (Chen and Trout, 1991; Chen et al., 1992).

Alginate systems have been used to produce restructured meats as an exclusive binder (Means and Schmidt, 1986; Means et al., 1987; Clarke et al., 1988a; and Clarke et al., 1988b). The advantage of an alginate system in general is that algin gels formed by the reaction of alginate and calcium salt at room temperature are heat stable and the gel is not thermoreversible. Therefore, restructured meats maintain their structural integrity through subsequent heating (Anonymous).

The most recent cold-set binder reported in the literature for restructured products is microbial produced transglutaminase (EC 2.3.2.13). Microbial transglutaminase can be used to bind various materials, such as meat, poultry, or fish, to any desired form without heating, freezing, and the addition of salt and phosphate (Sakamoto, 1994).

Calico scallop is considered as an underutilized species due to their small sizes. The annual U.S. scallop production from 1990 to 1996 showed that 4.7% of the mean production is calico scallops (*Argopecten gibbys)*, 1.13% bay scallops (*Argopecten irradians*), and 94.17% sea scallops (*Placopecten magellanicus)* (U.S. Department of Commerce, 1997).

The objective of this study was to study the effect of setting times on the physical properties of restructured scallops (*Argopecten gibbys*) produced with alginate and microbial transglutaminase.

Materials and Methods

Materials

Calico scallops (*Argopecten gibbys)* were purchased from the Kroger Company retail food store in Blacksburg, VA. Sodium alginate (Manugel® DJX) was provided by the Kelco Division of Merck & Co., Inc. (Chicago, IL). Sodium hexametaphosphate (Hexaphos®) was obtained from FMC Corporation, Chemical Products Group (Philadelphia, PA). Calcium sulfate dihydrate (Terra alba®) was purchased from the United States Gypsum Company, Chemical Division (Chicago, IL). Microbial transglutaminase (Activa ™ RM) was supplied by Ajinomoto U.S.A., Inc. (Teaneck, NJ). All ingredients used in the formulation were food grade.

Restructured scallops preparation

Small-sized calico scallops (1 kg) were thoroughly mixed in a bowl with a suspension of 10 g of microbial transglutaminase in 40 ml of cold water. The mixture was transferred into 3.175 cm (1 1/4 in) diameter cylinder molds. The molds were subsequently chilled at 5 °C for 2, 6, 9, 12, 18, and 24 hr for the cross-linking reaction to occur and then frozen at –20 °C. The frozen restructured scallops were removed from the molds, after 1 hr at room temperature and sliced into 1.905 cm (3/4 in) thick cylinders.

Sliced restructured scallops were thawed at room temperature for 2 hr prior to evaluating the physical properties by instrumental analyses.

For the alginate system, calico scallops (753.5g) were mixed with water (188.3g) and sodium hexametaphosphate (0.9g). A slurry of sodium alginate (7.5g in 18.8g of vegetable oil) was then added to the mixture and mixed until the gums were hydrated. A slurry of calcium sulfate dihydrate (7.5g in 23.5g of vegetable oil) was added and the mixing process continued until a uniform mixture was achieved. The mixture was immediately transferred into 3.175 cm (1 ¼ in) diameter cylinder shaped molds and subsequently chilled at 5 $^{\circ}$C for setting times of 2, 6, 9, 12, 18, and 24 hr and then frozen at –20 $^{\circ}$C. The restructured scallops were removed from the molds after tempering for 1 hr. at room temperature and sliced into 1.905 cm (3/4 in) thick cylinders. The sliced restructured scallops were thawed at room temperature for 2 hr prior to the physical property evaluation by instrumental analyses.

Evaluation of physical properties

The cylindrical specimens or restructured scallops from each treatment were compressed using the flat compression plate attached to the MTS® (Sintech 5/G, MTS corporation) crosshead. A 250 N load cell was used at a speed of 100 mm/min. The specimens were positioned on the stationary smooth surface under the flat compression plate. The dimensions of formed scallops were approximately 1.905 cm (3/4 inch) thick and 3.175 cm. (1 ¼ inch) in diameter. The exact dimensions of specimens used to

compute the stress and strain were measured with a vernier caliper before performing the tests. Stress-strain curve values were derived from the compression tests and used to calculate the physical properties. The calculated physical properties of scallops were: apparent modulus of elasticity, 5% secant modulus, 10% secant modulus, and 20% secant modulus. The definition of these physical properties followed those described by Mohsenin (1970).

Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA) with the Statistical Analysis System (SAS 6.12) to evaluate significant differences among treatments. Fisher's Least Significance Difference (LSD) test was used to determine differences between treatment means when analysis of variance indicated a significant difference at the $P \leq 0.05$ level.

Results and Discussion

Evaluation of physical properties

Mohsenin (1970) stated that a physical property measurement of a biological system such as plant and animal materials is a complicated situation due to their non-uniformity in nature and their susceptibility to change under prevailing environmental conditions. In other words, various factors affect the measurement of these materials. As long as no specific measurement was established for each type of material, only an empirical approach based on the application of fundamental principles of mechanics is possible to study the behavior of the materials under the applied forces. In this study, secant modulus and apparent modulus of elasticity derived from the stress-strain relationships were used to study the binding strength of restructured scallops.

Briefly, stress is expressed as force per unit of area (N/m^2 or Pa) and strain is the unit change in the size or shape of the body (mm/mm). Apparent modulus of elasticity is the slope before the initial yield point (the first stress in a material at which an increase in strain occurs without an increase in stress) on the stress-strain curve. Secant modulus is the slope of secant drawn from the origin to any specified point on the stress-strain curve (e.g. 5% secant modulus is the slope of secant drawn from the origin to 5% strain on the stress-strain curve).

Physical properties of restructured scallops produced by alginate and microbial transglutaminase investigated in this study were: apparent modulus of elasticity; 5%

secant modulus; 10% secant modulus; and 20% secant modulus. The physical property measurements of restructured scallops binding strengths are contained in Table 12 and 13.

From Table 12, the values of 5% secant modulus of restructured scallops with alginate gel at setting times of 2, 6, 9, 18, and 24 hr were not significantly different ($P>0.05$). Ten percent secant modulus with alginate gel setting time at 2, 6, 9, 18, and 24 hr were also not significantly different ($P<0.05$). The setting times of 2, 6, 9, 12, 18, and 24 hr did not affect the 20% secant modulus since there were no differences among the various setting times. These results suggest that secant modulus at 5, 10, and 20% strain were not appropriate measurements for the binding strength since the initial yield points did not occur at 5, 10, and 20% strain (0.05, 0.1, and 0.2 strain). The apparent modulus of elasticity measured before the initial yield point (the point that indicated the complete deformation of restructured scallops) was a good physical property indicator due to the differences found among the various setting times.

The setting times of restructured scallops produced with microbial transglutaminase did not affect the binding strength as measured by 5%, 10%, and 20% secant modulus. There were no significant differences ($P>0.05$) in 5% secant modulus, 10% secant modulus, and 20% secant modulus. In contrast, the apparent modulus of elasticity was significantly different at $P\leq0.05$ at various setting times.

The selected physical property used as a measurement of binding strength for both restructured scallops with alginate gel and microbial transglutaminase was the apparent

modulus of elasticity. Hereafter, the apparent modulus of elasticity will be referred as binding strength.

Effect of setting times on apparent modulus of elasticity (binding strength) of restructured scallops bound with alginate gel

The binding strength values of restructured scallops formed with 1% alginate gel at various setting times (2, 6, 9, 12, 18, and 24 hr) are given in Table 12. Significant differences ($P<0.05$) in the binding strength of restructured scallops formed with 1% alginate gel were obtained at various setting times. Increasing time from 2 to 24 hr had a negative effect on the binding strengths (Fig. 24). As the setting time increased from 2, to 24 hr, the binding strength decreased respectively from 158.7 to 75.0 kPa. The highest binding strength (158.7 kPa) was observed in restructured scallops after a 2 hr setting time. From these results, optimum gel strength is obtained with a short setting time. However, higher binding strength possibly occurs before 2 hr of setting.

The effect of setting time on the binding strength of restructured products was studied. Ben and Morris (1994) studied the effect of setting time on the binding strength of restructured beef steaks formulated with 0.4 and 0.6% alginate and set at 0 and 3 $^{\circ}$C for 6, 12, 18, and 24 hr. The highest binding (11 N) was obtained from meat with 0.6% alginate and set at 0 $^{\circ}$C for 18-24 hr. Increasing the setting time resulted in an increase of binding strength as measured by the force (N) required to push the ball through the slice (Field et al., 1984). Their results differed from our result in that our greatest binding strength (158.7 kPa or 1.587×10^3 N/m^2) was obtained from 1% alginate, a setting

temperature at 5 °C for 2 hr. The difference between our result and theirs came from the different concentration of alginate and setting temperature used to prepare the restructured product. Comparing alginate concentration, setting time, and setting temperature required to yield the highest binding strength between restructured meat (0.6% alginate, 18-24 hr at 0 °C) and restructured scallops (1.0%, 2 hr at 5 °C), restructured scallops are more economical to manufacture under commercial processing condition because of the relatively short setting time (2 hr) and environmental temperature (5 °C) required.

Effect of setting times on apparent modulus of elasticity (binding strength) of restructured scallops bound with Microbial Transglutaminase (MTGase)

The apparent modulus of elasticity (binding strength) of restructured scallops bound with 1% microbial transglutaminase at various setting times is given in Table 13. Significant differences ($P<0.05$) in the binding strength at different setting times were observed. Increasing time had a direct effect on the binding strength (Fig. 24), i.e., the binding strength increased significantly from 110.7 kPa to 336.0 kPa as the setting time increased from 6 to 24 hr. The binding strength of restructured scallops at 2 hr was not reported since no initial yield point was observed from the stress-strain curve.

The increased binding strength as time increased may result from the activity of microbial transglutaminase in the formation of a ε-(γ-glutamyl)lysine dipeptide. The dipeptide content in surimi gels with added 0.2% MTGase increased as a function of

setting time at 25 °C (Lee et al., 1997). From the same study, the content of ε-(γ-glutamyl)lysine crosslinks which was a direct evidence of transglutaminase activity correlated well with the gel strength (shear stress) and shear modulus at failure (Lee et al., 1997).

Conclusions

The cold-set binders, 1% alginate and 1% microbial transglutaminase (MTGase), used to produced restructured scallops at 5 °C at various setting times (from 2 to 24 hr) yielded different binding strengths as measured by the apparent modulus of elasticity. The highest binding strength of restructured scallops formed with 1% alginate occurred after 2 hr setting time. Increasing the setting time produced a negative effect on the binding strength of restructured scallops produced with 1% alginate as the binding strength decreased as a function of setting time. Conversely, increasing the setting time from 2 to 24 hr resulted in an increase in binding strength with 1% microbial transglutaminase. The highest binding strength with 1% microbial transglutaminase was observed at a setting time of 24 hr.

Although the setting time required to obtain the highest binding strength with MTGase (24 hr) was longer than alginate (2 hr), the binding strength of restructured scallops was much greater. The greater binding strength provides a greater benefit for consumer acceptability than the shorter setting time for the producer. Therefore, the recommendation for restructured scallops production is using 1%MTGase set at 5 °C for 24 hr.

Table 12: The effect of setting times at 5 °C on the physical properties: apparent modulus of elasticity, 5% secant modulus, 10% secant modulus, and 20% secant modulus of restructured scallops made from 1% (w/w) alginate gel.

Setting times (hr)	Apparent modulus of elasticity (kPa)	5% secant modulus (kPa)	10% secant modulus (kPa)	20% secant modulus (kPa)
2	158.7 ± 7.03 [a]	8.0 ± 4.51 [ab]	11.6 ± 5.01 [ab]	19.6 ± 6.00 [a]
6	118.3 ± 4.60 [b]	6.4 ± 5.37 [ab]	9.7 ± 5.44 [ab]	18.0 ± 6.18 [a]
9	114.9 ± 3.37 [b]	6.9 ± 2.66 [ab]	10.3 ± 2.41 [ab]	17.9 ± 4.31 [a]
12	93.9 ± 4.10 [c]	2.6 ± 2.59 [b]	7.6 ± 2.89 [b]	15.5 ± 3.02 [a]
18	90.7 ± 4.82 [c]	6.1 ± 5.64 [a]	10.8 ± 4.48 [a]	19.0 ± 4.21 [a]
24	75.0 ± 2.83 [d]	8.9 ± 2.25 [a]	14.9 ± 1.53 [a]	18.8 ± 1.20 [a]

[abcd] Means in the same column with the same letter are not significantly different (P>0.05) using One-way analysis of variance (ANOVA) with Fisher's Least Significance Difference (LSD) procedure.

Table 13: The effect of setting times at 5 °C on the physical properties: apparent modulus of elasticity, 5% secant modulus, 10% secant modulus, and 20% secant modulus of restructured scallops made from 1% (w/w) microbial transglutaminase (MTGase).

Setting times (hr)	Apparent modulus of elasticity (kPa)	5% secant modulus (kPa)	10% secant modulus (kPa)	20% secant modulus (kPa)
6	110.7 ± 10.79 [d]	18.4 ± 22.97 [a]	9.9 ± 6.03 [a]	18.6 ± 5.86 [a]
9	177.3 ± 8.56 [c]	8.7 ± 6.63 [a]	9.1 ± 7.66 [a]	20.0 ± 8.80 [a]
12	204.4 ± 30.17 [c]	6.5 ± 4.62 [a]	8.9 ± 7.55 [a]	17.7 ± 6.89 [a]
18	261.6 ± 9.17 [b]	6.9 ± 4.34 [a]	9.1 ± 4.25 [a]	14.7 ± 6.65 [a]
24	336.0 ± 27.59 [a]	6.9 ± 4.00 [a]	8.1 ± 2.41 [a]	20.1 ± 5.06 [a]

[abcd] Means in the same column with the same letter are not significantly different (P>0.05) using One-way analysis of variance (ANOVA) with Fisher's Least Significance Difference (LSD) procedure.

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APPENDIX I

Texture Profile Analysis (TPA)

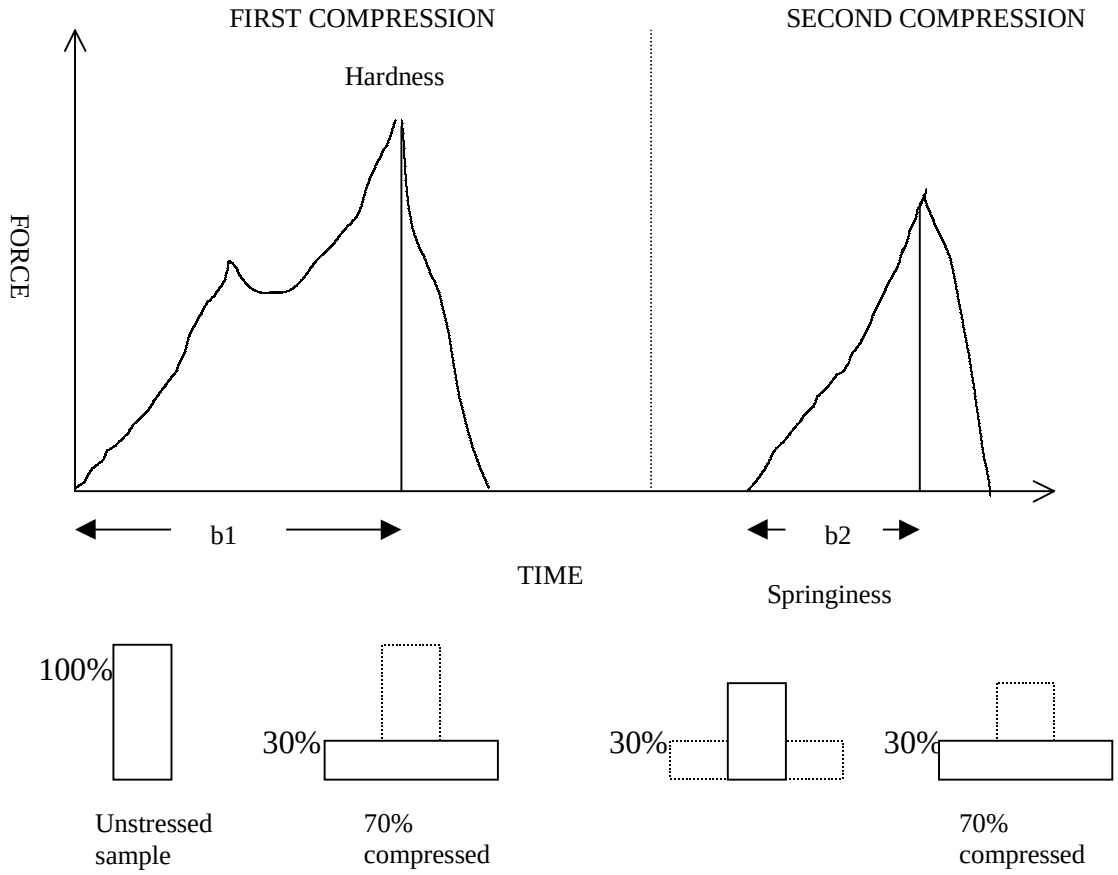

Fig. 14: The schematic of typical Texture Profile Analysis curves using an Instron Universal Testing Machine

Definition of Textural parameters

Hardness is the height of the force peak on the first compression cycle.

Cohesiveness (%) is the ratio of the total energy of the second compression and the total energy of the first compression times 100 (TE2/TE1 $\times$ 100).

Springiness (%) is the ratio of base width of second compression and base width of the first compression times 100 (b2/b1 $\times$ 100).

Description of Textural parameters

Hardness describes how easy to cut through the sample.

Cohesiveness describes the extent to which material can be deformed.

Springiness describes the recovery rate of material after deformation.

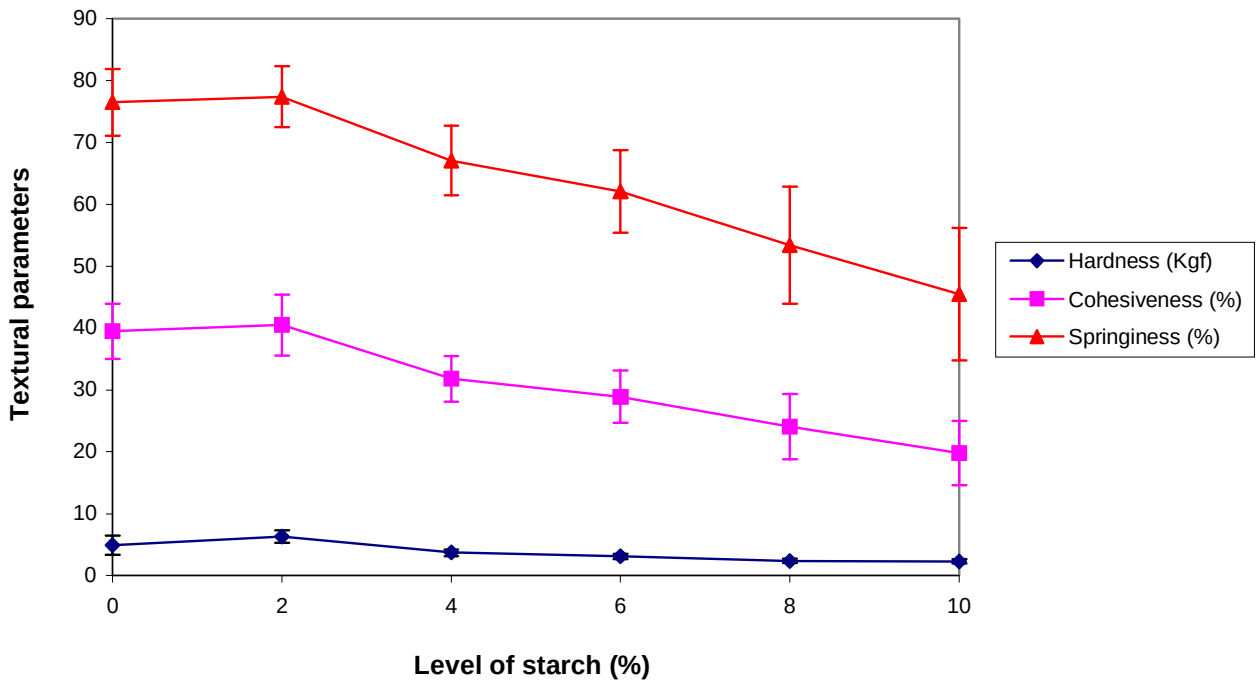

Fig. 15: The effect of 2, 4, 6, 8, and 10% starch on hardness, cohesiveness, and springiness of deep-fat fried restructured squid

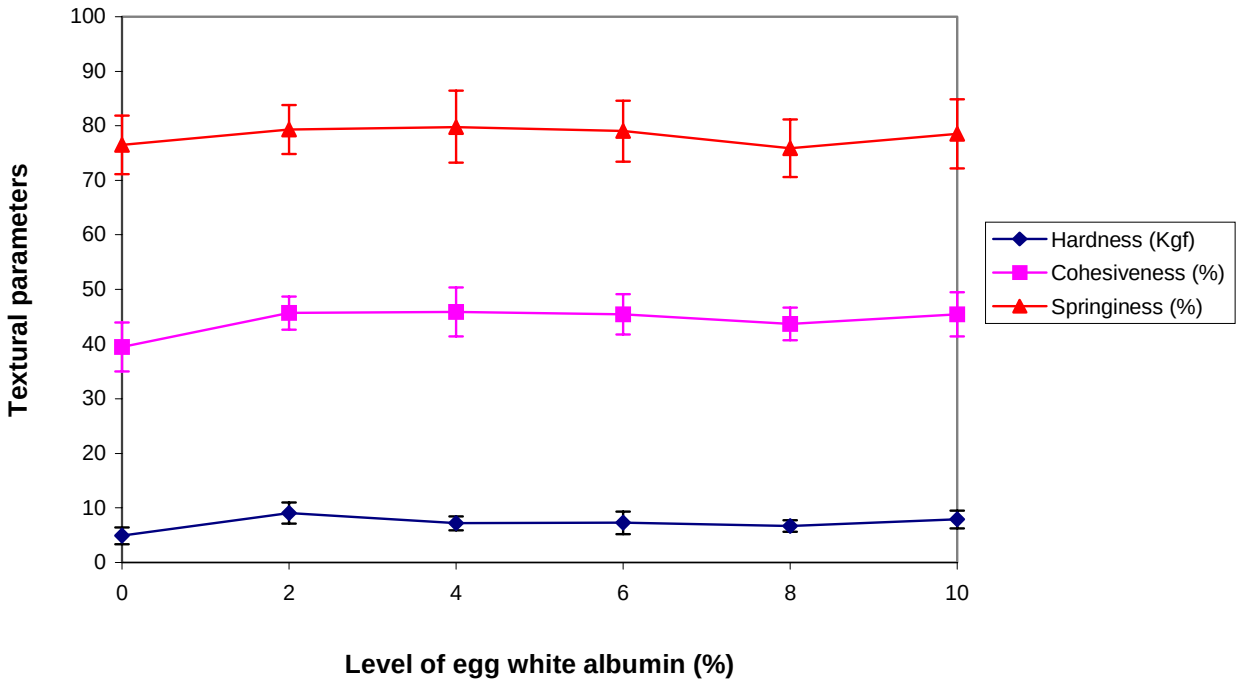

Fig. 16: The effect of 2, 4, 6, 8, and 10% egg white albumin on hardness, cohesiveness, and springiness of deep-fat fried restructured squid

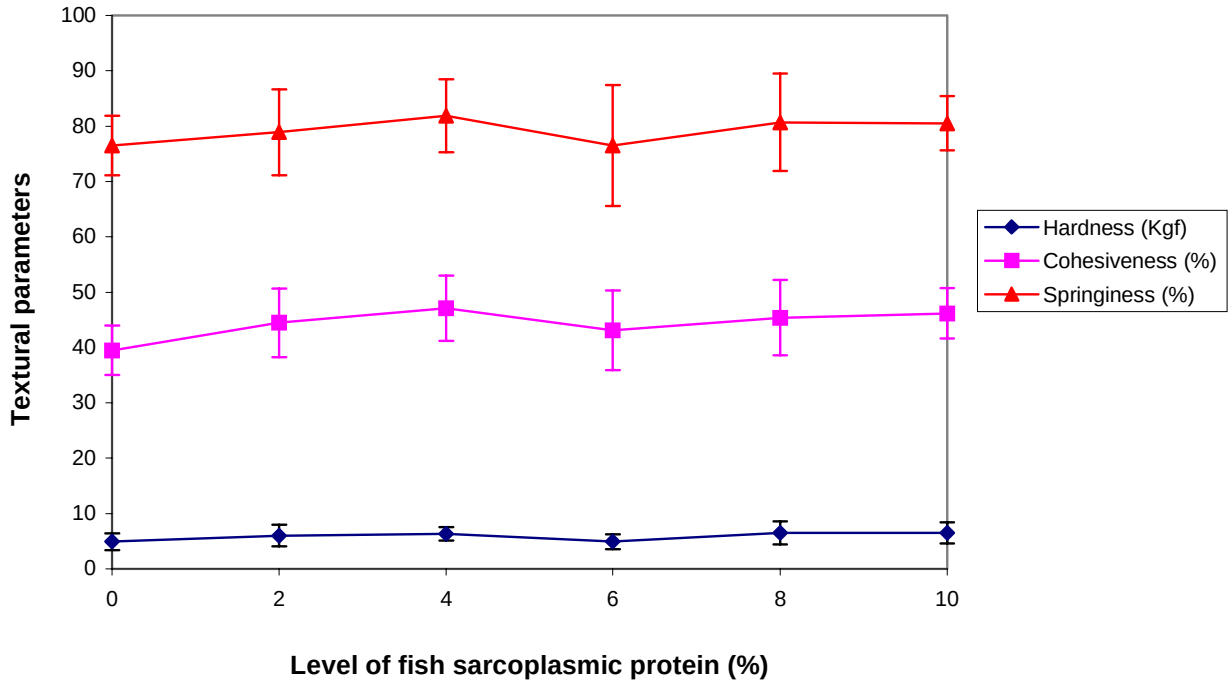

Fig. 17: The effect of 2, 4, 6, 8, and 10% fish sarcoplasmic protein on hardness, cohesiveness, and springiness of deep-fat fried restructured squid

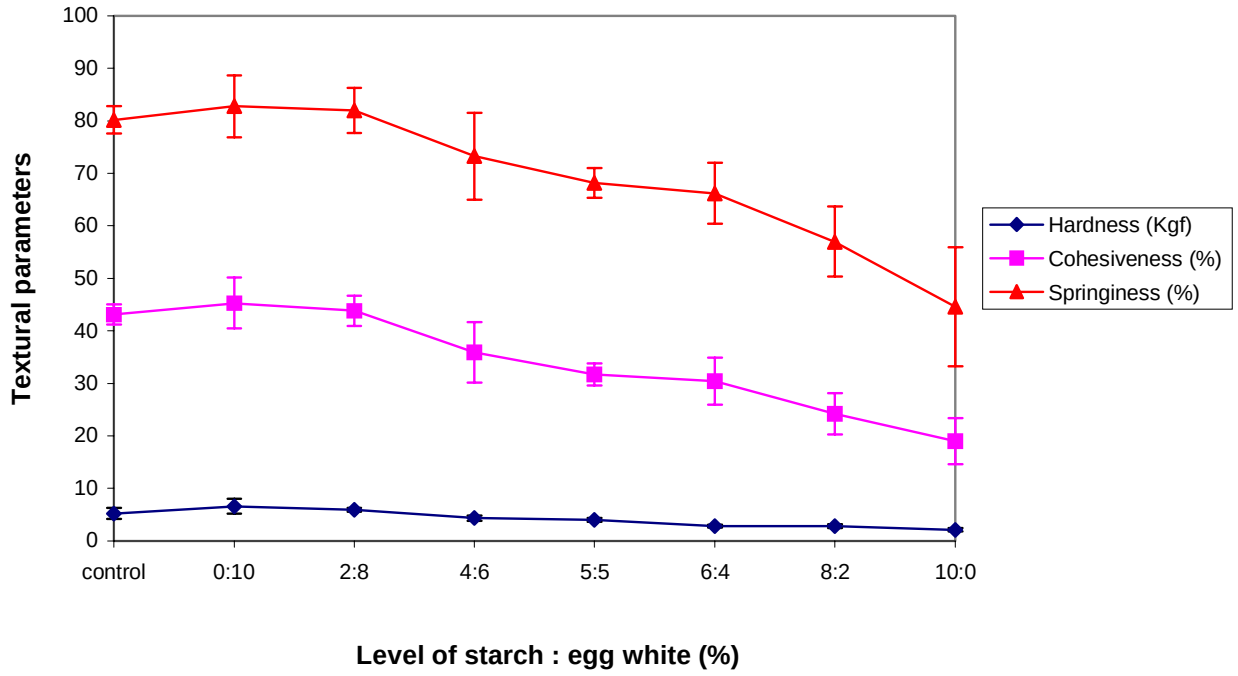

Fig. 18: The effect of starch:egg white (%) at 0:10, 2:8, 4:6, 5:5, 6:4, 8:2, and 10:0 level on hardness, cohesiveness, and springiness of deep-fat fried restructured squid

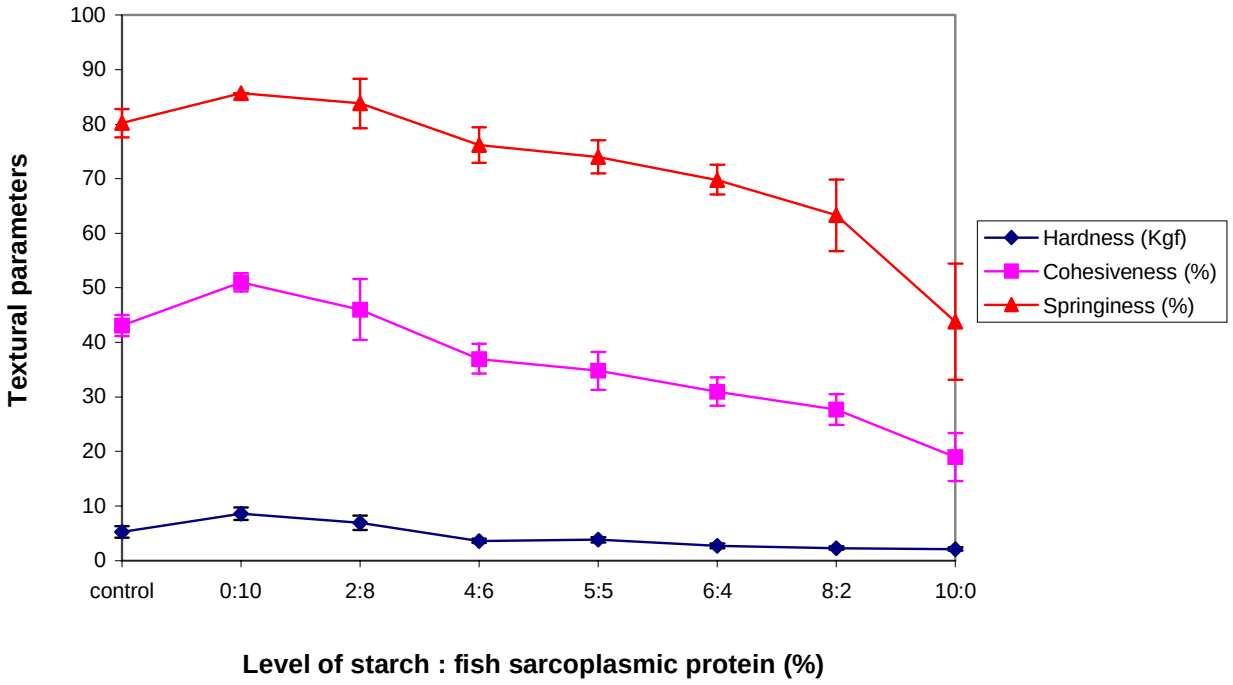

Fig. 19: The effect of starch:fish sarcoplasmic (%) at 0:10, 2:8, 4:6, 5:5, 6:4, 8:2, and 10:0 level on hardness, cohesiveness, and springiness of deep-fat fried restructured squid

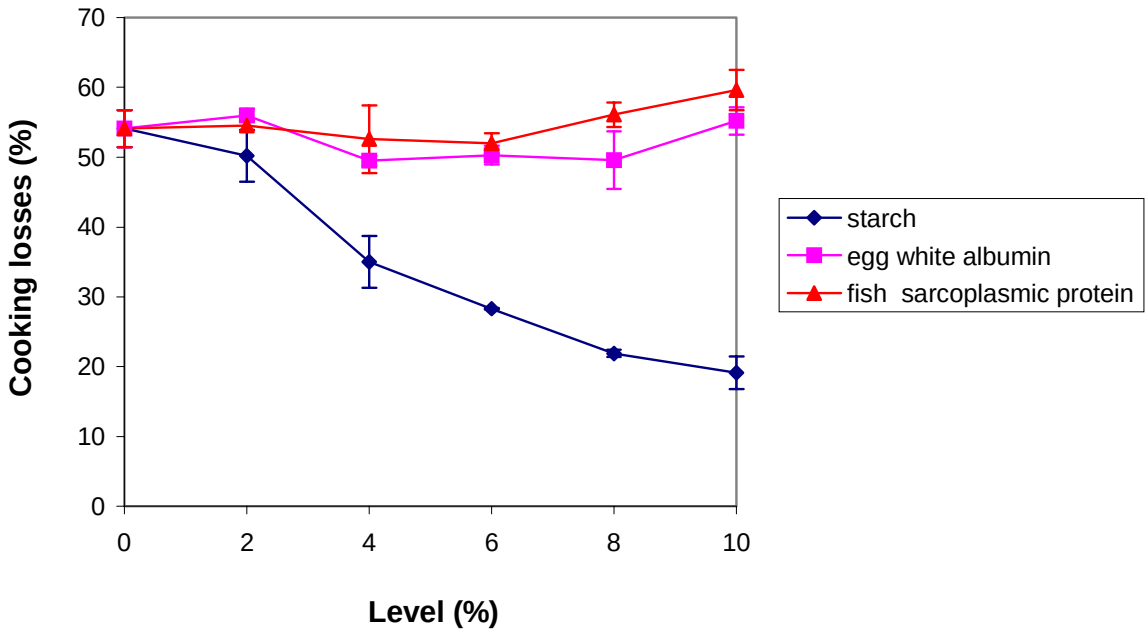

Fig. 20: The effect of 2, 4, 6, 8, and 10% starch, egg white albumin, and fish sarcoplasmic protein on the cooking losses of deep-fat fried restructured squid

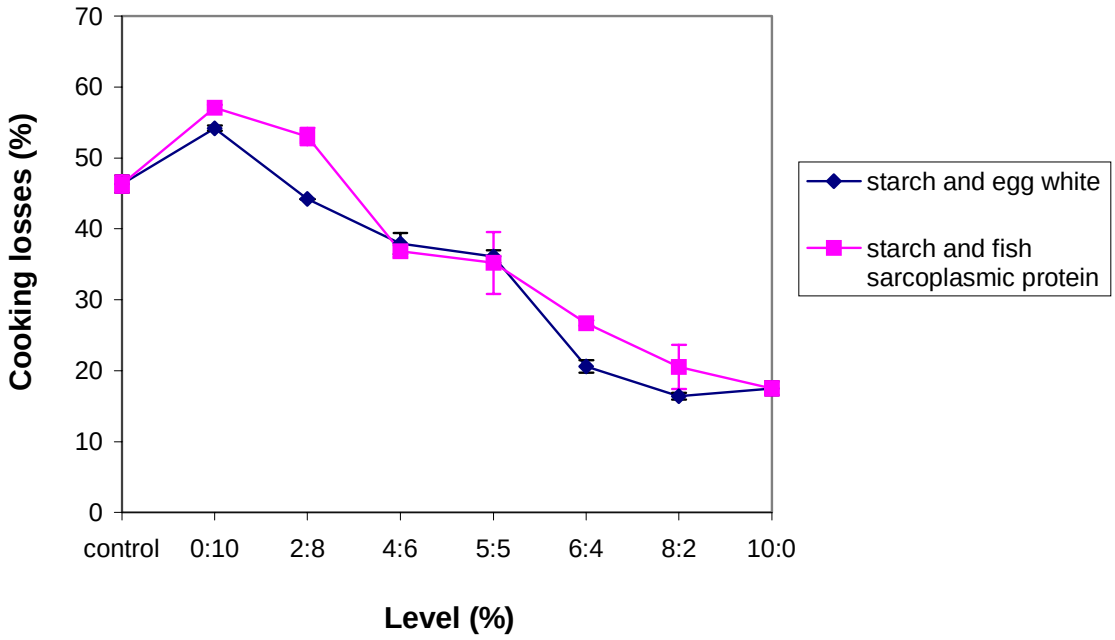

Fig. 21: The effect of combination between starch and egg white albumin and starch and fish sarcoplasmic protein at the combined level of 10% (0:10, 2:8, 4:6, 5:5, 6:4, 8:2, and 10:0) on the cooking losses of deep-fat fried restructured squid

APPENDIX II

The stress-strain curve

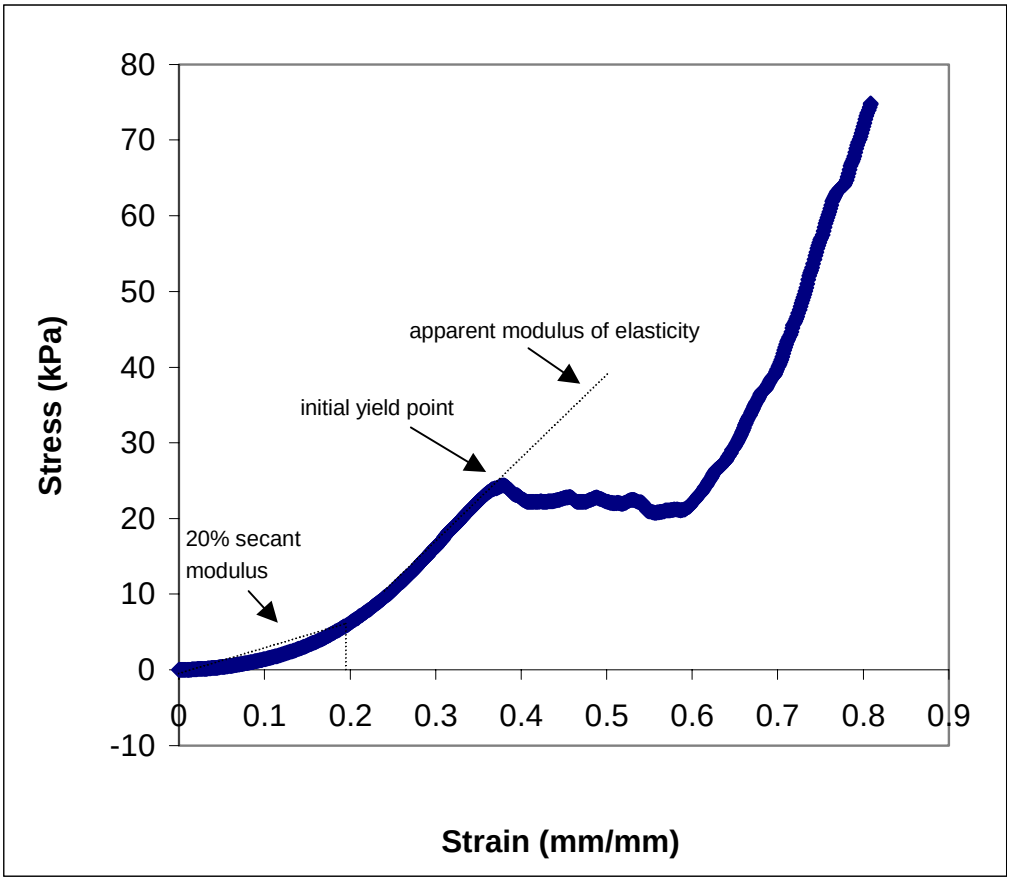

Fig. 22: The stress-strain curve of restructured scallops with 1% alginate set at 5 °C for 2 hr derived from compression test using MTS Universal Testing Machine at the crosshead speed of 100 mm/min.

Definition of physical property

Apparent modulus of elasticity is the slope of stress-strain curve before the initial yield point.

Secant modulus is the slope of the secant drawn from the origin to any specified point on the stress-strain curve.

5% secant modulus is the slope of the secant drawn from the origin to 5% strain on the stress-strain curve.

10% secant modulus is the slope of the secant drawn from the origin to 10% strain on the stress-strain curve.

20% secant modulus is the slope of the secant drawn from the origin to 20% strain on the stress-strain curve.

Description of physical property

Apparent modulus of elasticity is the binding strength of restructured scallops at the failure point of the gel bound pieces of scallops together.

Secant modulus is the binding strength of restructured scallop when they were compressed to any % strain. For example, 5% secant modulus is the binding strength of restructured scallops when they were compressed 5%.

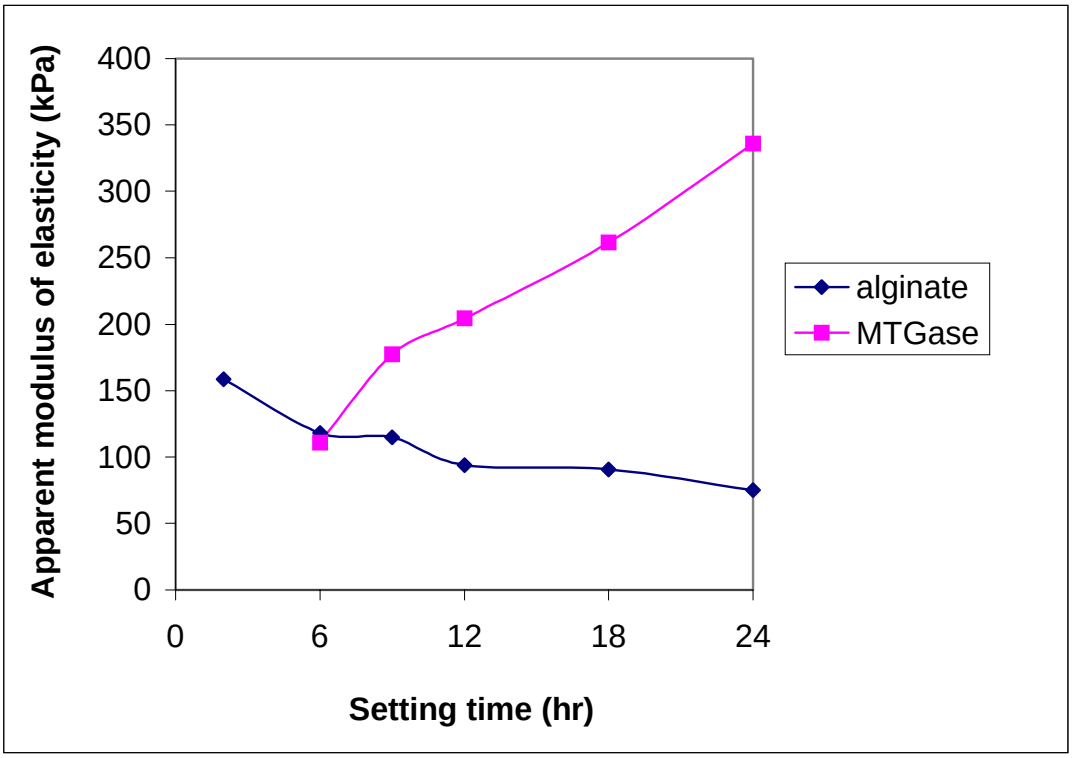

Fig. 23: The apparent modulus of elasticity (kPa) of restructured scallops bound with 1% alginate and 1% microbial transglutaminase (MTGase) at various setting times at 5 °C.

Vitae

Kannapha Suklim was born on June 6, 1973 in Bangkok, Thailand. She is the daughter of Mr. Sanam and Mrs. Buppha Suklim. After graduating with B.S. degree in Food Technology from Khon Kean University, she decided to pursue a M.S. degree in Food Science and Technology at Virginia Tech with a scholarship provided by the government of Thailand.

She entered Virginia Tech in the Fall of 1996 and pursued her graduate study under the direction of Dr. George J. Flick, Jr.