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THE DEVELOPMENT OF A PROCESS FOR THE PRODUCTION OF RESTRUCTURED FISH FROM RECOVERED FISH MINCE

A thesis presented in partial fulfilment of the requirements for the degree of Master of Technology in Food Technology at Massey University, Palmerston North, New Zealand.

Torben Sorensen

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Torben Sorensen

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SUMMARY

The edible flesh of fish can be recovered by meat/bone separating machines. These machines recover the flesh by tearing and shearing actions, forcing the flesh through a perforated drum in a form resembling minced meat.

A portion of the protein in fish flesh, as in meat and poultry, is salt soluble. If some of this protein is solubilised in the presence of salt, the protein forms a sol. When this sol is heated, a gel is formed. The gel is used in this development to hold the mince particles together, giving the product its structure. The structural characteristics of the gelled product are shown to be modified by adding varying amounts of salt, water and polyphosphate. These properties are applied to developing a heat gelled or restructured product with textural characteristics similar to cooked fish fillet.

The eating quality of the restructured product depends largely upon the quality of the raw materials used in its production. The quality of samples of fish is tested, and the heating operation used in the restructuring process is shown to reduce the number of bacteria in the product considerably. Quality standards are proposed.

Storage of product prepared from trevally is shown to be difficult due to the development of rancidity in fatty fish. The exclusion of oxygen from the product is found to be the most effective means of prolonging storage life.

A pilot plant is developed for the production of the restructured product. The restructured product is modified to suit the continuous process, whilst retaining the desired textural characteristics. A manufacturing process is proposed, its operation based on the pilot plant.

SECTION I

Introduction

Recovery of edible flesh by meat/bone separators

The production of fish paste products such as Kamaboko (fish paste) and chukawa (fish sausage) is an ancient tradition in Japan, and it is only recently that the industry has developed into a mass production enterprise (Tanikawa 1963). To produce the quantity of boneless, ground fish required for this industry, the Japanese have developed a range of machines by which the material can be processed, and an appropriate technology to ensure quality standards are met. These developments have proved to be very successful, and the principles developed by the Japanese are now being adopted by a number of industries throughout the world.

The success of the meat/bone separation machines is largely due to their versatility, and the high yields of edible fish flesh that can be recovered. Using this process most species of fish will yield 55% of edible material (King and Carver 1970), although flat fish may yield as little as 25% (Tanikawa 1963) and species such as Blue Marlin may yield up to 70% edible fish (King and Carver 1970).

Working with fish caught off the coast of New Zealand, Sorensen (1971b), found that the yield of edible fish flesh recovered depended on the species of fish, and varied seasonally. In every case it was shown that processing the fish by machines yielded more edible fish than traditional processing methods (Table 1:1):

TABLE 1:1 Comparison between yields of machine recovered fish and fish filleted by hand

Species	Machine Recovery (%)	Hand Operation (skilled knifehand)	
		fillet recovery (%)	Skinned & Boned fillet (%)
Snapper	50 - 54%	31 - 37%	25 - 32%
Gurnard	51 - 53%	40 - 44%	-
Trevally	52 - 56%	42 - 46%	19 - 22%
Barracautta	50 - 54%	40 - 44%	-

In addition to the high yields of edible materials recovered from headed and gutted fish, work has also been done to recover edible flesh from fish frames or fish scrap remaining after filleting. Sorensen (1972) reported that an additional 19% could be recovered from snapper by processing the frames after filleting. King and Carver (1970) reported similar results from a variety of fish including ocean perch, pollock and cod.

The high yield of edible flesh recovered from all species of fish, and the ability of the machines to process a wide variety of fish without special fixtures or adjustments has already had an impact on the New Zealand industry. Two companies are currently using meat/bone separators to process species of fish which would otherwise be under utilised. Barracautta, previously difficult and uneconomic to process, largely due to its bone structure, is now used in the production of slabs for use in fish finger processing (Slack 1973). Similarly, recovered trevally fillet is used in products such as fish cakes (Sorensen 1971b).

It is not known how much fish has been dumped overboard

from New Zealand fishing vessels, although it was reported in 1966 (Anon., 1966) that as much as 200 tons of trevally, kahawai and red cod were dumped overboard each month. In addition to the dumping of commercially recognised fish is the dumping of fish caught incidental to the main catch. A survey by a Japanese research vessel off the east coast of New Zealand showed that a number of fish not used commercially at present were suitable for Surumi (ground fish) production.

Uses for Ground Fish Flesh

Although a market does exist for Surumi in Japan, current prices are not attractive, and the quality demanded by Japanese importers would require the Surumi to be processed only from premium quality fish. Thus uses must be found for the product either in New Zealand or Australia.

Ground fish flesh has been used in fish cakes, canned fish products (King and Carver 1970), fish luncheon sausages, savoury fish bites and fish burgers (Sorensen 1971b). The material has also been used to produce the fish sausage described by Thomas (1966) and in Surumi-type fish fingers (Achjolberg 1971, Slack 1973). In addition it has been used as a binder in fish loaf or jellied roll type products (Learson et al 1969, King and Carver 1970) and has been used in the preparation of simulated shell fish products (Learson et al 1971).

Sandwich, or hors d'oeuvres type of spreads have also been proposed, and King and Carver (1970) cite a case for institutional or commercial mass feeding, where the aim is to produce a nutritive menu at a low cost. These workers claim that preliminary tests suggest that it is possible to include freshly prepared machine separated fish flesh with ground beef in recipes

such as hamburgers, sloppy joes, meat loaf and spaghetti with meat sauce. A beefless frankfurter was also proposed.

Marketing of Processed Seafood Products

Market surveys in New Zealand (Nielsen 1971) and Australia (van Dijk 1971) on frozen fish products indicate a strong demand for fish fingers (fish sticks) and fish portions. In view of the U.S. fish stick production increase of 16.9% in 1972 (Q.F.F. 1973) it would appear that this trend is likely to continue.

Compounded products such as fish cakes on the other hand, appear to have more limited appeal. This can be seen in Table 1:2, listing local retail sales of fish fingers, frozen fish fillets and fish cakes for the twelve months ending July 1971. (Other figures for New Zealand are not available, and the local sales trend is not known).

TABLE 1:2 Sales of frozen uncanned fish products in New Zealand; for 12 months ending July 1971

Product	Sales Volume (lbs.000)	Retail value ($.000)	% Of Grocery Turnover
Fish fingers	1404.7	1093.3	0.45
Fish fillets	427.3	335.8	0.14
Fish cakes	85.6	56.0	0.02

(Condensed from Nielsen Report, 1971)

Having established the importance of the fish finger and the frozen fish fillet market, it is necessary to understand why consumers prefer these products. A survey carried out by Heylen Research Centre (1972) showed that people have an image of what a fish finger should be, and have expectations associated

with this image. It appears that a fish finger should contain "white flesh", and have a "real fish flavour", and a "moist" and "fine" texture. Similar criteria can be expected for all products traditionally processed from natural fish fillet. Fish fingers can be processed directly from frozen slabs of ground fish (Sorensen 1971b, Schjolberg 1970, and Slack 1973), and such products are currently sold on the New Zealand Market (Slack 1973).

Consumer reaction to fish fingers processed from ground fish was evaluated from a survey conducted by the Heylen Research Centre (1972) on behalf of JBL Seafoods Limited.

Three fish fingers were evaluated on a comparative basis:

(1) Smoked fish fingers prepared from ground fish; fish content 72%

(2) White fish finger prepared from ground fish; fish content 72%

(3) White fish finger prepared from fish fillet; fish content 50%

Results from this survey suggest that the smoked fish finger did not have the characteristics expected of fish fingers. This product was commonly associated with fish cakes. A comparison between the two white fish fingers revealed the overriding importance of textural characteristics in fish products. Although the fish fingers prepared from ground fish were preferred for their flavour, the overall preference was with the product prepared from natural fish. The appeal for this product was largely due to its texture, which was described

as "real fish" with a "light", "fine" texture. In addition the texture was noted to be "moist", "juicy" and "succulent". The products prepared from ground fish on the other hand, were described as "tough", "chewy", "heavy" and "dry". Some people disliked the product being "minced", and others considered the product to be "like sausages".

An indication of the value placed on the texture of the product was found by a "propensity to purchase" test carried out during the survey. It was shown that most housewives considered fish fingers prepared from fish fillet to be a better buy at 69c per 10 oz pack, than fish fingers prepared from ground fish selling at 58c for a 10 oz pack.

Products prepared from skinned and boned fish fillet pose a number of problems to the producer. Skinning and boning fish is a slow and laborious process, and during the peak of the season, factories do not have the time or the staff to handle large quantities of fish in this way. Furthermore, the low yields and high labour content result in high costs, and despite the premium paid for products prepared from skinned and boned fillets, the proposition is not financially attractive.

Restructured Flesh Products

The foregoing discussion outlines the problem faced by the fishing industry, and it is noted that the use of the meat/bone separation process to recover high yields of edible flesh can be of great benefit to the industry. On the other hand, it is also appreciated that products prepared from fish fillets are most desirable from a marketing point of view. To satisfy both of these requirements, it would be necessary to reprocess the recovered flesh to simulate a form that resembles

and has the textural qualities associated with fish fillet. For the purpose of this thesis, such a process will be referred to as "restructuring".

The principle of restructuring flesh materials depends upon the binding together of individual particles to form a continuous mass. The binding is achieved by dissolving a portion of the myofibrillar proteins by the action of salt (sodium chloride), and forming a "sol", which is very adhesive (Tanikawa 1970). When this adhesive mixture is heated, the sol converts to a gel, forming a ternary network protein structure enclosing particles of ground fish.

Using this principle, a number of different concepts have been proposed in the literature on restructuring flesh products. Unilever (1971) in a patent application for restructuring ground fish, meat and poultry, describe a system whereby fibrous material is laid parallel and intimately bound together by the addition of comminuted meat components. The textured component contains separate meat muscle fibre bundles (length 2 - 50 mm), fat (1 - 40%), salt, water and additives such as herbs, farinaceous or other vegetable material and vegetable protein. The alignment of the textural components is achieved by extrusion with orientation of fibre bundles and is consolidated by freezing or heat coagulation.

Work has also been done on the binding together of cubes of meat. Schnell et al (1970) found that cubes of chicken could be bound together by the action of heat to form chicken loaves. These workers found that the amount of fluid expressed during cooking was inversely related to the amount of binding

achieved. Sodium chloride and food grade phosphate compositions were reported to reduce the fluid expressed, and thereby assisted the binding properties of the meat cubes. A process of tumbling meat cubes has also been described (Anon. 1971). Tumbling the meat is said to produce optimum protein activation, resulting in excellent binding and reduce cooking losses. Sommer (1969) describes a process in which pieces of moist meat are worked for 20 - 25 minutes, to release protein substances to the surface of the meat where binding is effected. This process has also been used by Sorensen (1972) in the preparation of fish finger slabs. Torr (1970), Gilbert (1971) and Wilcox and Hafstead (1969) all describe variations of the process whereby salt is used to extract myofibrillar protein from pieces of animal or poultry meat, causing them to become tacky, and then forming the pieces into a coherent mass and coating with an external binder.

Fenters and Ziemba (1971) describe a process in which boneless meat is cut into flakes instead of being ground, the flakes being knitted together subsequently during blending. The blended meat is frozen, tempered, then machine pressed into loaves ready to be sliced or cubed. The authors claim that the restructured meat can be used in various forms, including stews and casseroles, and that it will not disintegrate on cooking.

Processes have also been described where introduced binders are used to hold together the pieces of meat. Binders proposed include fish protein (Learson 1971), plant protein, caseinate, skim milk powder and egg albumin (Torr 1970).

SECTION II

Theory on the Nature of Binding in Meat Particles

Note: The term "meat" is used in this section for the sake of continuity with published work. The principles discussed apply equally to meat, fish and poultry.

The binding of meat is a heat initiated reaction, as meat in its raw form does not show binding to any extent (Vadehra and Baker 1970). Hamm (1966) studied the changes which occur in meat protein during cooking, and found that the helical portion of the protein molecules in meat unravel to form randomly organised chains. These chains become linked by the formation of hydrogen and ionic bonds. In this work Hamm (1966) reported that the nature of the coagulum formed depends on the pH and ionic strength of the medium. Added phosphates are also known to affect binding, and their addition to meat products may increase or decrease the binding strength, depending on the salt concentration in the product.

Where it is desired to increase the binding strength in meat products beyond that which can be achieved by gelling the naturally soluble meat protein, binders such as starch (Tanikawa 1970) and vegetable proteins (Vadehra and Baker 1970) have been used.

To understand the nature of the reactions which occur during the formation of meat gels in the binding process it is necessary to be familiar with the composition and the structure of meat and meat proteins.

Composition of Meat

Skeletal muscle contains approximately 76% water, 18%

protein, 3% fat, 1% glycogen and 2% salt (Lawrie 1966). The muscle proteins are further divided into the myofibrillar and sarcoplasmic proteins, and the connective tissues as shown in Table 2:1.

The structure of meat

(a) Macro-structure

The essential unit of all muscles is the fibre. The muscle fibres are held in place by a system of connective tissue. Surrounding each fibre is the endomysium, connective tissue less than 5 microns thick (Bendall 1965). The endomysium fuses into the perimysium which groups the fibres into bundles. The size of the muscle fibre bundles determines the texture of the muscle. (Hammon 1932, Wills 1960 cited Lawrie 1966). In muscles capable of finely adjusted movements, as in those which operate the eye, the muscle fibres are fine, whereas in those performing grosser movements the bundles are large.

Muscle bundles are in turn held in place by the fusion of the perimysium with the epimysium, a heavy sheath of connective tissue which surrounds the muscle as a whole. The epimysium blends into massive aggregates of connective tissue, known as tendons, by which the muscles are attached to the skeleton.

Connective tissue includes formed elements and an amorphous ground substance of mucopolysaccharide in which the formed elements generally are embedded.

The formed elements consist of (a) fibres of collagen, which are straight, inextensible and non-branching; (b) elastin, which is elastic, branching and yellow in colour; and (c) reticulin, which resembles collagen, but is associated with

TABLE 2:1 Chemical composition of typical Adult Mammalian Muscle after Rigor Mortis but before Degradative changes Post-Mortem (per cent Wet Weight) (After Lawrie 1966)

Water			%	75.5%
PROTEIN	Myofibrillar	(myosin, tropomysin, (X protein	7.5	18.0%
		(actin	2.5	
	Sarcoplasmic	(myogen, globulins	5.6	
		(myoglobin	0.36	
		(haemoglobin	0.04	
	Mitochondrial	(cytochrome C	ca0.002	
	Sarcoplasmic reticulum Sarcolemma Connective tissue	(collagen) (elastin) ("reticulin") (insoluble enzymes)	2.0	
Fat			%	3.0%
SOLUBLE NON PROTEIN SUBSTAN-CES	Nitrogenous	(creatine	0.55	3.5%
		(inosine monophosphate	0.30	
		(di- and tri- (phosphopyridine (nucleotides	0.07	
		(amino acids	0.35	
		(carnosine, anserine	0.30	
	Carbohydrate	(lactic acid	0.90	
		(glucose-6-phosphate	0.17	
		(glycogen	0.10	
		(glucose	0.10	
	Inorganic	(total soluble (phosphorous	0.02	
		(potassium	0.35	
		(sodium	0.05	
		(magnesium	0.02	
		(calcium	0.007	
		(zinc	0.005	
Traces of glycolytic intermediates, trace metals, vitamins, etc.			ca0.10	

substantial quantities of a lipid containing myristic acid.

(b) Micro-Structure

Muscle cells, or muscle fibres, are long, narrow, multinucleated, and may stretch the entire span of the muscle, attaining a length of 34 cm, although they are only 10-100 µm in diameter (Walls 1960, cited Lawrie 1966). Surrounding each cell, and lying beneath the endomysium is a sheath, called the sarcolemma, a double membrane structure, with membranes 50-60 Å apart (Robertson 1957, cited Lawrie 1966). Within the sarcolemma sheath are the myofibrils which are surrounded by a fluid phase, the sarcoplasm. In the sarcoplasm are found certain formed structures, the mitochondria or sarcosomes, the sarcoplasmic lipid bodies and the sarcoplasmic reticulum or tubular system, as well as dissolved or suspended substances of the glycolytic cycle. The muscle cell nuclei are generally found just beneath the sarcolemma.

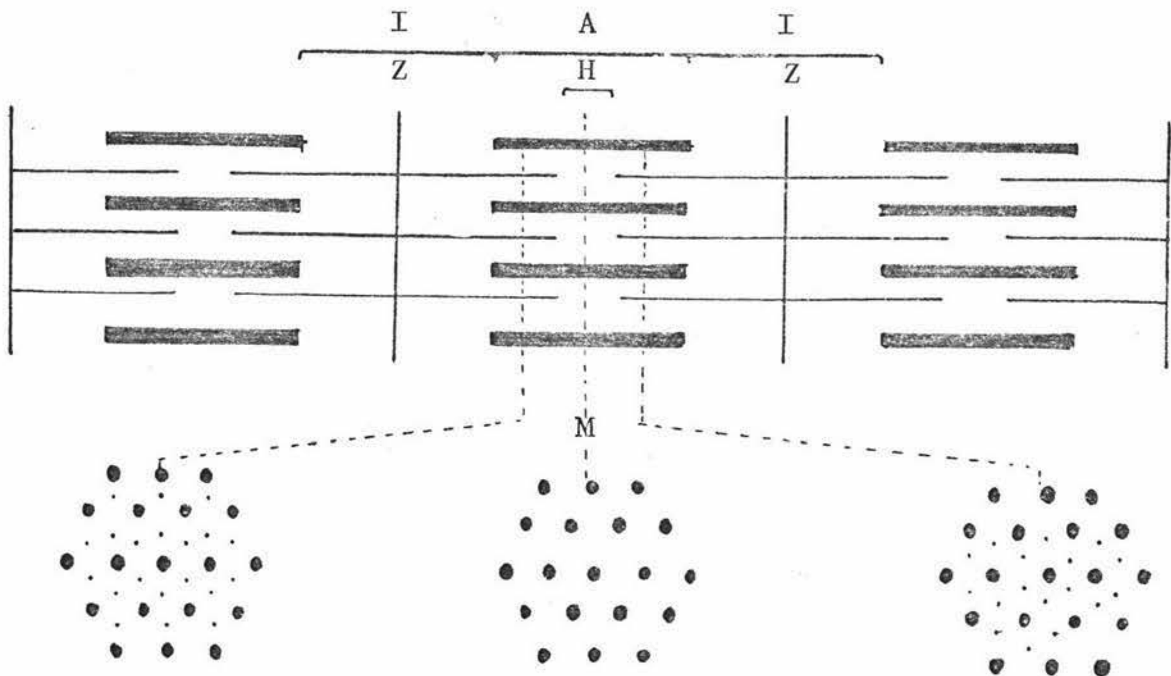
The structure of the muscle fibre is such that the myofibrils, 1 or 2 microns thick, (Bendall 1965) are separated from one another by the fine network of tubules of the sarcoplasmic reticulum, which meet at points along each fibril to form the so called triads, from which transverse tubules pass from one fibril to another, until they come in contact with the sarcolemma. The sarcoplasmic reticulum transmits chemical changes arising as a result of nervous impulses from the sarcolemma to the individual myofibrils.

The myofibrils are considered to be composed of two sets of filaments, each forming a hexagonal array, running parallel to the axis of the muscle fibre (Gergely 1970) as

shown in Figure 2:1. The distance between two adjacent Z lines is the functional unit of the myofibril and is known as the sarcomere (Lawrie 1966).

FIGURE 2:1 Above: Schematic representation of three sarcomeres in L.S. as seen by electron microscope. Thick lines are myosin filaments and the thin lines actin filaments.

Below: Diagramatic representations of T.S. through the A branch at three points, one being the H zone (taken from Lawrie 1966)



The thick set of filaments are called myosin filaments, and are built up of myosin molecules, arranged head to tail as shown in Figure 2:2. The heads, which are made of heavy meromyosin, appear on the filament as projecting feet with a sixfold screw axis of symmetry, whereas the tails of light meromyosin serve to bind the filaments together.

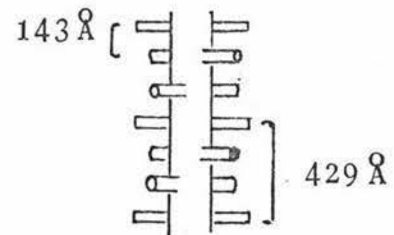
In the filaments, the feet are 429 Å apart in each row. At the centre of each myosin filament in the A band, there is a bare patch without feet, and it is here that the

backbone molecules of light meromyosin from each half filament are bound together (M band) (Bendall 1966).

FIGURE 2:2 Aggregation of myosin and arrangement of heavy meromysin projections

(a) Schematic representation of filament formation. Note reversal of polarity on either side of the centre (cited Gergely 1970; from Huxley 1963)

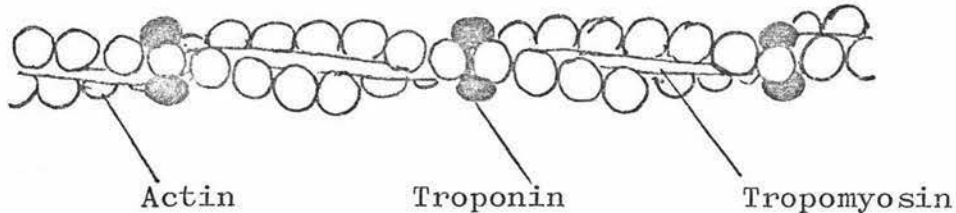
(b) Helical arrangements of projections on myosin filaments deduced from X-ray data (cited Gergely 1970; from Huxley and Brown 1967).



The thin sets of actin filaments with a thickness of 70 Å were once thought to consist purely of a double chain of globular G-actin monomers, helically arranged so that the pitch of the helix is about 700 Å, there being 13 monomers per turn with a spacing of about 55 Å (Bendall 1965, 1966). More recent work by Ebashi, Endo and Ohtsuki (1969) has shown that tropomyosin and troponin also are contained in the structure of the actin filaments at the so called Z line. The actin filaments from each half sarcomere are joined together in this region in a complex criss-cross fashion (Bendall 1966), to give the greater density observed at this point on electron micrographs.

A model for the fine structure of the filament is shown in Figure 2:3.

FIGURE 2:3 A model for the fine structure of the thin filament (cited Maruyama and Ebashi 1970)



Properties of Muscle Proteins

The proteins of muscle can broadly be divided into three groups: those which are soluble in water or dilute salt solutions (sarcoplasmic proteins) those which are soluble in moderately concentrated salt solutions (myofibrillar proteins) and those which are insoluble in concentrated salt solutions, at least at lower temperatures (proteins of connective tissue and other formed structures).

The sarcoplasmic proteins

The sarcoplasmic proteins (myogen and globulins) are now known to represent a complex of about 50 components, many of which are enzymes of the glycolytic cycle. Most of these components belong to the class of albumins (Bendall 1965), have isoelectric points between pH 6 and 7, and molecular weights in the range of 30,000 to 100,000. Many of them are easily denatured under mild acid conditions (pH 4.5).

The Myofibrillar proteins

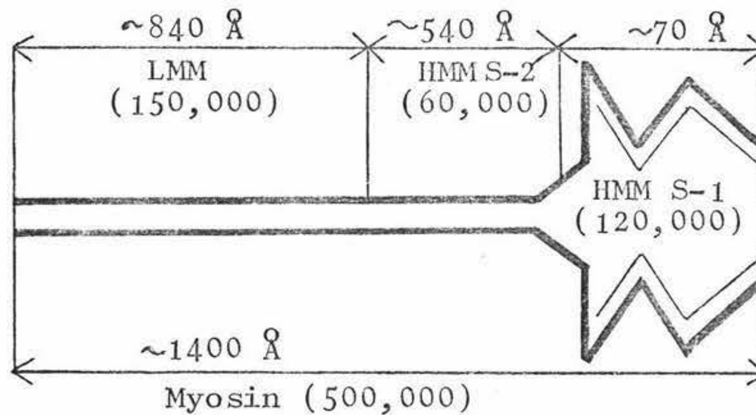
(a) Myosin

Briskey and Fukazawa (1972) describe the myosin molecule as a slightly rigid rod, approximately 1600 Å in length, and 20 Å in diameter, with a thickened bulbous end of somewhat variable diameter, although most reported values are in the range of 30-60 Å. The tail of the molecule appears to be a helical coil of two-polypeptide chains. The two super-coiled L-helical segments of this tail of the molecule stabilize each other by strong side chain interactions along most of their length.

Myosin has for the past twenty years been regarded as being composed of two major fragments; the light (LMM) and heavy (HMM) meromyosins. These products can be formed by splitting the peptide bonds near the centre of the rods by tryptic digestion (Gergely 1950; Szent-Gyorgyi 1953, cited Briskey and Fukazawa 1972).

The Keilly-Harrington model (1960) which proposed that the molecule consisted of three-polypeptide chains folded over at the end where the molecule is thickened has been questioned by Perry (1967). More recently Lowey, Slayter, Weeds and Baker (1969) showed that the myosin molecule consists of two major polypeptide chains which run the entire length of the myosin molecule (Figure 2:4). Proteolytic enzymes are able to split the complex at two points, thus producing the original L-helical fragment, L-meromyosin, a second highly helical fragment, subfragment-2, and two rather globular particles that carry the adenosine triphosphatase activity and the ability to combine with actin.

FIGURE 2:4 Schematic representation of the myosin molecule. The numbers in parenthesis indicate molecular weights. Two light chains are indicated, one adjacent to each HMM S-1. (Cited Gergely 1970)



The adenosine triphosphatase enzyme carried in the HMM S-1 is activated by calcium ions, and inhibited by magnesium ions. The ATPase activity has pH optima of 6.4 and 9.2, activity of the latter being nearly twice as great as the former. These enzymatic properties are sensitive to changes in temperature and in pH, and can be modified by the addition of a variety of reagents.

(b) Actin

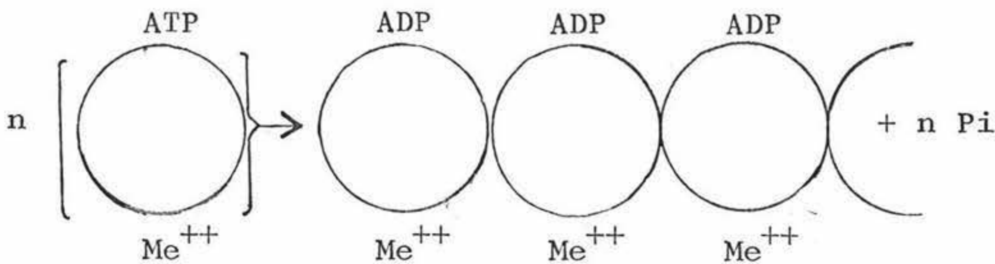
Globular actin (G-actin) has a molecular weight of about 50,000 and has the shape of a sphere (Briskey and Fukazawa 1972). The molecule appears to be about 55 Å in diameter and contains approximately 450 amino acid residues.

G-actin contains one molecule of ATP and one mole of tightly bound divalent metal - per mole of monomer. The molecule of ATP is available to enzymes and readily exchanges with ATP of the medium. The divalent metal may be either Ca or Mg, or may be a mixture of both. Evidence suggests that the presence of the nucleotide (ATP) and the divalent metal

contribute to the stability of globular form.

On the polymerization of actin monomers to form the fibrous aggregate of F-actin, ATP is dephosphorylated to ADP with the liberation of phosphate. As shown in Figure 2:5, the degree of polymerization is directly proportional to the moles of ATP dephosphorylated.

FIGURE 2:5 Polymerization of G-actin on addition of salts



The nucleotide and the metal ion are exchangable in the globular form, but quite stable, both kinetically and thermodynamically, in the fibrous form. The roles of the nucleotide and the bound metal are not clear as polymerization of actin can take place in vitro in the absence of one or both of these.

(c) L-actinin

L-actinin was originally discovered by Ebashi and Ebashi (1965), and has since been found to consist of light, intermediate and heavy components with sedimentation coefficients of 6, 10 and 28 s respectively (Briskey and Fukazawa 1971). The work of Briskey et al (1967) and Musaki et al (1967) indicates that L-actinin is probably located at the Z band and appears to be associated with the extensive cross-linking found at that point (Briskey and Fukazawa 1971). In support of the possible associative effects, it has also been shown that L-actinin binds to F-actin and L-actinin tightens

the suspension of F-actin during superprecipitation.

(d) Tropomyosin

Tropomyosin, which was discovered by Bailey (1946, 1948), has similar solubility properties to myosin, and the two proteins are also similar in amino acid composition and in their iso-electric points (Briskey and Fukazawa 1971). Tropomyosin resembles LMM in structure, being close to 100% α-helical. The main difference between myosin and tropomyosin is that the latter has no ATP-ase activity.

Briskey and Fukazawa (1971) reported that tropomyosin extracted from rabbit is characterised by the following:

(1) The formation of highly polymerized viscous solutions at neutral pH and in the absence of salts.
(2) Reversible depolymerisation upon the addition of dilute salts.
(3) Molecular asymetry exceeding 20.
(4) Resistance to the denaturation effect of organic solvents and acid pH.

Leki _et al_ (1962) showed that tropomyosin is bound to F-actin in an approximately stoichiometric ratio. It has also been shown that the viscosity of F-actin is greatly influenced by the addition of tropomyosin (Drabikowski and Gergely 1962).

(e) Troponin

Troponin and tropomyosin are now known to form a complex previously called "native tropomyosin". Among the structural proteins, troponin is the only one with the ability to bind Ca^{++}, a capacity that is not modified by the presence

of tropomyosin, F-actin, myosin or ATP (Briskey and Fukazawa 1971). Tropomyosin and troponin are complexed with G-actin in the formation of the thin F-actin filaments. Troponin can also modify the F-actin structure but only indirectly through the mediation of tropomyosin as discussed in the next section.

Hartshorne and Mueller (1968, 1969) separated troponin into A and B fractions. According to these workers troponin A is the Ca^{++} receptive protein and B is an inhibitor of the Mg^{++} activated adenosine triphosphatase activity of actomyosin.

Interaction of the Myofibrillar Proteins

(a) Muscle functions in vivo

It has been well established that the interaction of myosin and F-actin in the presence of Mg ATP plays an essential role in the molecular mechanism underlying muscular contraction (Maruyama and Ebashi 1971). Nervous stimulation of the sarcomere is transferred via the tubules to the sarcoplasmic reticulum which releases Ca^{++} ions thus initiating a sequence of reactions which cause myosin and F-actin to interact.

Originally it was thought that the interaction of myosin and F-actin was directly influenced by the ability of Ca^{++} ions to free ATP from its magnesium complex, and to activate myosin-ATPase (Lawrie 1966). It has now been found that the contractile process is regulated directly or indirectly by the so called "regulatory proteins", troponin and tropomyosin (Maruyama and Ebashi 1970). These workers found that in the absence of Ca^{++}, troponin exerts an inhibiting effect on the

interaction of F-actin and myosin in the presence of Mg ATP. It is now considered that the release of Ca^{++} from the sarcoplasmic reticulum removes this inhibition, thus allowing the interaction to occur between F-actin and myosin, when activated by the splitting of ATP and ADPase.

In addition to its direct regulatory function, it is suggested that troponin also controls the interaction of myosin and F-actin by modifying the structure of F-actin (Ebashi and Endo 1968; Ebashi, Kodama and Ebashi 1968). It appears that troponin undergoes a Ca^{++} dependent conformational change which effects a modifying action on F-actin structure in the presence of tropomyosin.

After contraction, relaxation normally occurs. Whereas the contractile proteins of muscle containing adequate Ca^{++} (bound on troponin) contract in the presence of ATP and ATPase; it has been found that if part of the Ca^{++} is removed, relaxation occurs (Marsh 1961). The removal of the Ca^{++} is controlled by the "relaxing factory", a function organised as an aspect of the sarcoplasmic reticulum, and sometimes referred to as the Marsh-Bendall factor after Marsh (1952). It has been suggested that in controlling the environmental Ca^{++}, the relaxing factor indirectly controls the amount of Ca bound to the thin myofibrillar filaments (Marsh 1966).

More recently relaxation has been described in terms of an antagonism between myosin and troponin (Webber 1970). According to this author, the degree of antagonism is dependent on the binding of Ca^{++} to troponin on one hand, and to the binding of ATP to myosin on the other. When the ATP concentration is so low that the binding of ATP to myosin is less than

20% of the maximum possible there is no antagonism, and independent of the Ca^{++} concentration, relaxation cannot occur.

Over the range of increasing ATP saturation of myosin (2-50 μM) the interaction with actin decreases to a minimum if troponin is free of Ca^{++}; but the binding of only 1 Ca^{++} ion per troponin molecule is effective in overcoming this inhibition of interaction. Under conditions where myosin is fully saturated with ATP, however, two sites available on the troponin molecule for Ca^{++} ion complexing must be saturated to overcome the antagonism and to allow any significant activation of myosin adenosine triphosphatase by actin to occur.

(b) Post Mortem changes

Consideration of post mortem change will be confined to modifications which occur in the myofibrillar proteins during the conditions commonly referred to as rigor mortis and "ageing". For an overall view of post mortem change, the reader is referred to Bendall (1960), Cassens (1966); Newbold (1966); Lawrie (1966) and Goll et al (1970).

Rigor mortis has been described as the condition in the post mortem muscle which causes the shortening or contr-action of the muscle fibre. The shortening of muscles on opposite sides of the same bone results in a stiffness or rigidity in the carcass (Goll et al 1970), and has been observed to coincide with the depletion of ATP and the formation of lactic acid due to the anaerobic glycolytic cycle (Bendall 1960, Lawrie 1966). After the ATP falls to 10-20% of its initial level, irreversible actomyosin bonds are formed

(Bendall 1960) and the muscle becomes inextensible (Goll <u>et al</u> 1970).

Shortening of post mortem muscle is caused by the contraction of a relatively small proportion of the myofibrils (Bendall 1960), and the isometric tension lasts only while ATP is available as an energy source. The degree of shortening is important when muscle is considered as a source of food, because it is related to the tenderness of the meat (Lawrie 1966). In muscles which pass through rigor mortis in an extended condition, myofibrillar filaments overlap and cross-bond at fewer points, and the amount of actomyosin formed is small. Such meat is tender on cooking. On the other hand when the degree of shortening is high, and the muscles pass through rigor mortis in a contracted condition, there is much cross-bonding and the meat is relatively tough on cooking.

The degree of shortening is known to be temperature dependent, and meat passing into rigor mortis at low temperatures is known to undergo more severe shortening. Under conditions of extreme shortening, the water holding capacity of the meat may also be adversely affected (Lawrie 1966).

The inflexible state of the muscles, ultimately achieved on the completion of rigor mortis has been ascribed to the cross linkages between actin and myosin filaments in the absence of ATP. Newbold (1966) suggested that under post mortem conditions, the nature of these cross linkages is different to the contractile bonds in vivo. More recently Goll <u>et al</u> (1970) suggests that the troponin-tropomyosin complex is either broken down during rigor mortis, or the complex in the post

mortem form is unable to confer Ca^{++} sensitivity on the actin-myosin interaction. It is inferred that without the regulatory proteins, the actomyosin reaction cannot be controlled, and relaxation of actomyosin is not possible.

Ageing, which follows the onset of rigor mortis, results in the muscle becoming increasingly pliable, but remaining inextensible (Lawrie 1966). Earlier work in this field (Lawrie 1966) suggested that these changes were due to the effect of proteolytic enzymes still active in the muscle, and due to bacterial spoilage. More recently it has been reported (Goll et al 1970) that the myofibrillar proteins undergo at least two specific alterations during post mortem storage; (1) A loss of Z-line structure, and in some cases, complete removal of both the Z-line and the M-line, and (2) modification of the actin-myosin interaction. The suggestion that the actin-myosin interaction is modified is supported by the evidence that ageing results in a 20-80% increase in the actomyosin adenosine triphosphatase activity, and at the same time, results in the increase sensitivity of the actin-myosin complex to dissociation by ATP. This suggests that pyrophosphates, which have the ability to increase the water holding capacity in meat by the dissociation of actomyosin (in a similar way to the dissociation by ATP), would be most effective when used in properly aged meat.

(c) Interaction in Isolation

1. Myosin

Extracted and purified myosin is known to undergo two distinct types of aggregation (Perry 1967), depending on the ionic strength and the pH of the medium.

(i) The aggregates are formed spontaneously at a molecular concentration of less than 0.35M. This is a reversible process, and the aggregates are several molecules thick, and may be many molecules long. The size of the aggregate is dependent on the pH and the ionic strength.

(ii) Aggregates are also formed spontaneously at a high molecular concentration (above 0.5M) in the physiological pH range, but appear to be irreversible in nature. This process is probably associated with tertiary structural changes and the loss of biological activity. The aggregates are generally much smaller than those formed at low ionic strength.

2. Actin

The addition of salt to G-actin effects immediate and rapid aggregation of the monomers to form F-actin. The transformation of G- to F-actin appears to take place by the polymerization of a globular molecule into a linear aggregate with the form of a double helix (Briskey and Fukazawa 1971). The strands are intertwined in a regular manner, so that cross over points occur at 360-370 Å intervals. Although troponin and tropomyosin have been shown to associate with actin in the natural fibre, they do not appear to be required for polymerization to occur.

The rate of polymerization is governed by the salt concentration, and the degree of polymerization is inversely regulated by the salt concentration. Thus the velocity of

of polymerization is maximal at a potassium chloride or a sodium chloride concentration of 0.1-0.15M, a further increase in the concentration of the salt causes a decrease in the extent of polymerization, until eventually it is abolished.

3. Actin-Myosin

The interaction between actin and myosin can assume various forms, depending on the conditions under which the proteins are combined. Under conditions of high salt concentration - about 0.5M potassium chloride - there is an increase in viscosity (Bendall 1965, Gergely 1970, Briskey and Fukazawa 1971); at low salt concentration (0.1-0.15M), resembling that prevailing in living muscle - a fine suspension is formed (Gergely 1970).

Briskey and Fukazawa (1971) consider the viscosity change as the establishment of cross-bridges between actin and myosin filaments, whilst Bendall (1965) attributes the high viscosity formed in the presence of a high ionic concentration to the formation of the so called arrowhead structure. In this model it is envisaged that the change in properties, on the formation of actomyosin, is due to the bonding of the HMM heads of the myosin molecules to each pair of monomers along an F-actin chain of indefinite length, with the LMM tails of the myosin molecules sticking out into the solution, giving a high viscosity, and a wide range of molecular weights.

The reaction involving the formation of actomyosin is endothermic (Briskey and Fukazawa 1971), and the viscosity of actomyosin decreases as the temperature falls. In addition it has been demonstrated (Wood 1951) that superprecipitated actomyosin dissolved more rapidly at $0^{o}C$ than at $25^{o}C$.

When the salt concentration is reduced to below 0.15M, solutions of actomyosin form gels (Bendall 1965). On the addition of ATP to the gel, the immediate effect is the classical superprecipitation, first observed by Szent-Gyorgyi in 1942.

ATP has a two-fold effect when added to the gel; the first, to loosen the actomyosin bonds and to allow myosin and actin molecules to slip freely past one another; that is, a plasticizing effect; the other, as an energy source for the physical change during superprecipitation, when ATP is converted to ADP by the enzymatic centres on myosin. For the plasticizing effect alone, Mg ions are obligatory and Ca ions must be rigidly excluded. For the superprecipitation effect, traces of Ca ions must also be present to activate the enzymatic centres, allowing the free energy of the splitting process to be used in the physical change.

Relationships between Binding Quality of meat products, and the Myofibrillar Proteins

The importance of the myofibrillar proteins to binding, or to the tensile strength produced in heat coagulated meat gels, was clearly established by Fukazawa *et al* (1961 a, b) who found that the removal of actomyosin from a model muscle system resulted in a gel formation with no binding quality. Conversely, it was found that the removal of the water soluble proteins from the model did not greatly affect the binding strength. These findings were supported by Nakayama and Sato (1971 b), who reported that the reduction of the actomyosin content in experimental gels corresponded to a weakening in gel strength.

The binding properties displayed by actomyosin are thought to be largely due to the myosin fraction. Sato and

Nakayama (1971 b) noted that the binding quality of heat set actomyosin gel increased with myosin concentration. Fragmentation of myosin revealed that heavy meromyosin (HMM) had little influence on heat gelling in systems composed of stroma and myofibrillar proteins, also light meromyosin on its own (LMM) showed no effect on the heat-gelling properties. This suggests that the whole molecule of myosin is required for the development of the binding properties and that the use of proteolytic enzymes in the production of heat bound products should be avoided.

Fukazawa et al (1961 a) found that F-actin itself exerts little influence on the heat-gelling properties of a system, although when in the form of actomyosin it appeared to be useful in stabilizing the system. This is supported by Samejima et al (1969) who found that F-actin improved the gel strength when complexed to myosin. Nakayama and Sato (1971 b), working with actin/myosin models, also found that the binding quality was dependent on the ratio of actin to myosin. In addition, these workers found that the binding quality was increased by the addition of tropomyosin to the system.

Role of Sodium Chloride in the Binding of meat systems

Salt (sodium chloride) has been found to be an essential ingredient in products such as sausages (Lawrie 1966), kamaboko (Tanikawa 1963, 1971) and reconstituted products which depend on the presence of actomyosin for their binding characteristics (Sommer 1969, Torr 1970, Galbert 1971, Wilcox and Hafstead 1968). The role of salt in such systems is to solubilize and effect the extraction of the myofibrillar proteins from the intracellular milieu. On the basis of the manufacturing process

for sausages it may be supposed that only a portion of the myofibrillar proteins are dissolved before heat processing. The sausage texture will then depend on the solubility and extractability of the salt soluble proteins (Samejima <u>et al</u> 1969). In most processing operations, the extraction of the salt soluble proteins is assisted by mechanical agitation such as tumbling, mixing and grinding.

In addition to the formation of gels necessary for binding, the incorporation of salt in comminuted meat products is important in enhancing the water holding capacity (Lawrie 1966). The binding strength of the gel formed on heating and the water holding capacity are very closely correlated (Vadehra and Baker 1970), suggesting that both properties are dependent on the formation of the salt - actomyosin complex.

The mechanisms by which salt solubilizes the myofibrillar protein is not altogether clear. Work done on isolated systems (foregoing discussion) indicates that whole myosin must be solubilised and that actin is involved in the form of a stabilizer. Ohashi <u>et al</u> (1970) suggest that on the addition of salt, metal ions are partially released from the muscle proteins and the steric structure of the protein is relaxed. This could help to explain the high osmotic pressure which results when salt is added to muscle tissue (Lawrie 1966).

<u>Effect of Phosphates</u>

A number of workers (Nakayama and Sato 1972, Fukazawa <u>et al</u> 1961 c, Vadehra and Baker 1970) have reported that the addition of phosphates have a beneficial effect on the binding strength of meat gels. Fukazawa <u>et al</u> (1961 c) found that the

effect of phosphates was dependent on the presence of salt. It was concluded that the ionic strength in cured meat maintains a condition such that the muscle structured protein is drawn out through the sarcolemma of the muscle cell, and that such action may be promoted by the action of phosphates.

The effect of phosphate has also been found to be dependent on the condition of the myofibrillar proteins. That is, in the native state, the effect of phosphates is to assist in the extraction of the overall content of the myofibrillar protein. On the other hand, if the proteins are denatured, the effect of phosphate is due only to an increasing extraction of light meromyosin (LMM). As LMM does not greatly influence the binding strength in heat gelled systems, it is suggested that if the proteins are denatured, phosphates may benefit the water holding capacity, but not the binding strength of the gel.

In a comparison between the effectiveness of phosphates, Fukazawa _et al_ (1961 c) found that only pyrophosphates had the ability to solubilize denatured fibrils. In natural fibrils it was found that pyrophosphate had the greatest effect, and that tripolyphosphate was more effective than hexametaphosphate.

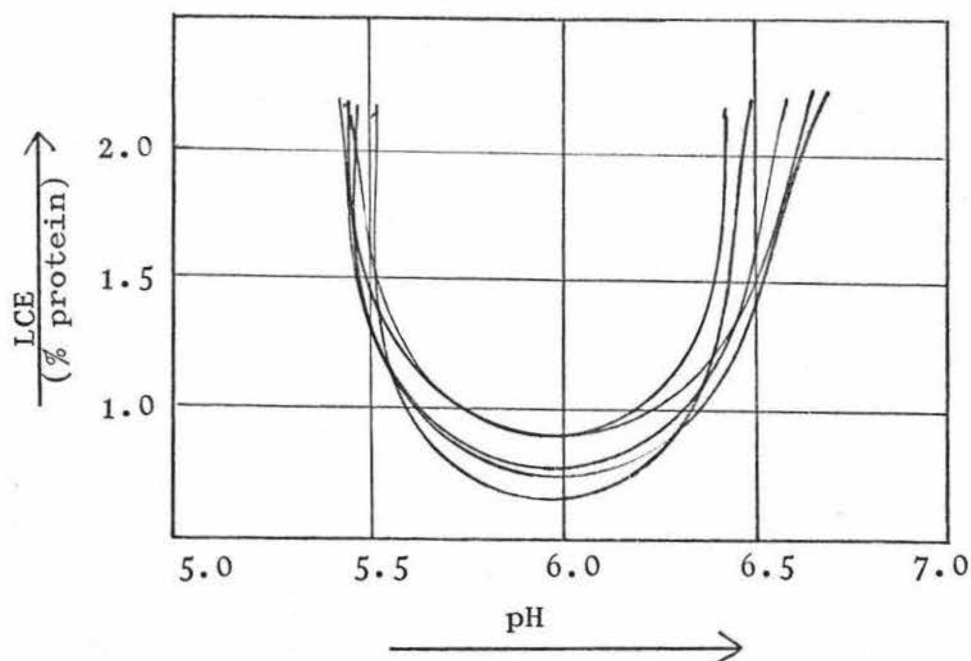
Bendall (1954) suggested that the effect of most phosphates is largely due to one of an ionic strength and pH; whereas that of pyrophosphate was due to the splitting of actomyosin into actin and myosin. This view has been confirmed by Fukazawa _et al_ (1961 c) who found that actomyosin was dissociated by the addition of pyrophosphate, but not by the addition of other phosphates. The ability of pyrophosphates to split actomyosin has also been reported by Nakayama and Sato (1972), who found that the effect of pyrophosphate was similar

to that of ATP. When either of these components were added at pH 7.0 to unheated minced meat sol, the binding strength was increased in the heated gel, as shown by an increased strain at the breaking point and an increased hardness of the gel.

Effect of pH on binding quality

The effect of pH is similar to that of both salt and polyphosphate in that binding is dependent on the solubilization of the myofibrillar proteins. Using the Least Concentration End point (LCE) Trautman (1966) found that the relationship between pH and the binding gel strength characteristics is highly significant. The results obtained with pre rigor porcine muscle are plotted as a family of curves (Figure 2:6) representing five heat treatments from 18°C to 41°C, and show that the best LCE was found between pH 5.7 and 6.0.

FIGURE 2:6 Effect of pH and temperature on the Least Concentration End point of a pre rigor percine muscle extract containing water soluble and salt soluble proteins; heat treatment for 30 min.



Similar results were obtained for post rigor muscle, where the optimum LCE was found between pH 6.0-6.3. This indicates that there is an optimum pH range in which soluble proteins give a more rigid gel. This conclusion was also reported by Vadehra and Baker (1970), who suggested optimal binding values for poultry meat were between pH 6.0 and 7.0.

Heat Coagulation of myofibrillar proteins

The colloidal-chemical changes observed during heating are mainly a result of the coagulation of myofibrillar proteins. Hamm (1966) suggests a mechanism of heat denaturation of muscle tissue as follows:

Between 35°C and 50°C the actomyosin molecules are unfolded. At about 35°C coagulation begins, caused by the aggregation of the unfolded molecules and accompanied by the formation of new relatively unstable cross linkages. Between 50°C and 70°C the unfolding as well as the coagulation continued. In this temperature range a tight protein network linked by relatively stable bonds is formed. This process is possibly due to new hydrogen bonds, to some disulphide - sulfhydryl exchange, and/or to interactions between nonpolar side chains.

Although unfolding and coagulation are closely related, it should be noted that in not every case does unfolding of the peptide helix proceded the coagulation.

Changes relating specifically to the alteration of a salt solubilized solution to a stable gel upon heating could not be cited, although it is possible that the mechanism described above could occur in solutions, and thereby form a network structure of protein molecules as described by Tanikawa (1971).

SECTION III

Experimental Work on Binding in Restructured Fish

Introduction

Edible fish is recovered by the meat/bone separator in the form of shredded flesh and has an appearance similar to mincemeat. The machine removes the flesh from suitable materials by pressing and tearing actions, forcing the flesh through the perforated drum. The effect of this action is to rupture a proportion of the cellular material, squeezing out large quantities of moisture and soluble substances, including proteins, salts and blood pigments.

The fish mince recovered by this process may therefore be regarded as a dispersion of small discrete particles of flesh, surrounded by a fluid phase. If the product is steamed in this form, no binding occurs, and free moisture collects in the bottom of the cooking vessel.

When the mince is beaten, as in a high speed mixer, the external phase becomes tacky. In this form free moisture is bound in and the dispersed particles become more difficult to separate out, apparently because of the binding of the external phase on to their surface. Heating the product in this form results in the external phase forming a gel, which binds the product together.

The work reported in this section deals with the binding characteristics of heat gelled fish mince. The effect of mixing in various amounts of salt, phosphates and water to the mince prior to cooking are evaluated from studies of the mechanical properties of the gelled product.

Fish Materials

Much of the experimental work reported in this section was carried out from June to August, and fresh fish could not be obtained. Thus, a large proportion of the fish used was drawn from frozen stock processed some six months previously in Auckland, and transported to Massey University by refrigerated transport. This was not always satisfactory, and on a number of occasions the fish sent down for the experimental work was lost en route, or arrived in an unusable condition.

The effect of this is that some of the experiments were carried out using trevally and the rest using gurnard. The results indicate what fish was available at the time.

Although actual results varied between the two species as shown in Table 3:2 the changes due to different treatment were consistent.

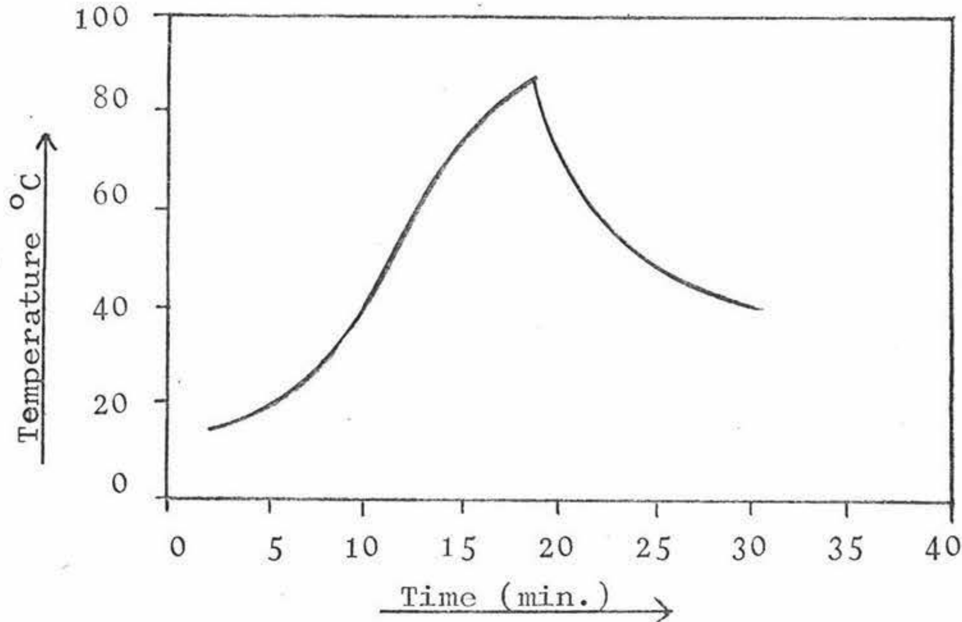
Preparation of Samples

The samples were based on 150 g fish mince, other ingredients being added as indicated in each experiment. Each sample was mixed for 90 seconds at high speed in a Kenwood Chef mixer using a "K" beater. The mixture was then filled into a 90 mm diameter glass petri dish, and allowed to equilibrate for 24 hours at $0^{o}C$ in the chiller. Following this the samples were cooked by steaming at atmospheric pressure ($100^{o}C$) for 20 minutes.

The internal temperature during steaming was measured using a Varian millivolt recorder, with the thermocouple fitted through a hole drilled in the petri dish with the junction

placed in the centre of the sample. The temperature profile for this steaming process is given in Figure 3:1.

FIGURE 3:1 Temperature profile, showing the temperature of the centre of a test sample during steaming



After cooking, the sample was allowed to cool, then stored in the chiller overnight before testing.

The tests were carried out in triplicate on plugs taken from the sample with a No. 12 cork borer. The plugs were cut to a height of $\frac{1}{2}$ in. before testing. Thus the test sample was the shape of a cylinder 0.5 in. high and 0.7 in. in diameter.

Test Equipment

The textural characteristics of each sample were evaluated by compression test using an Ottawa Texture Measuring System (OTMS). This system of texture measurement is based on an electrically powered press fitted with a force transducer to detect the force necessary to deform a sample. The transducer output is passed on to an amplifier which in turn feeds the signal

FIGURE 3:2 Schematic diagram of the Ottawa Texture Measuring System

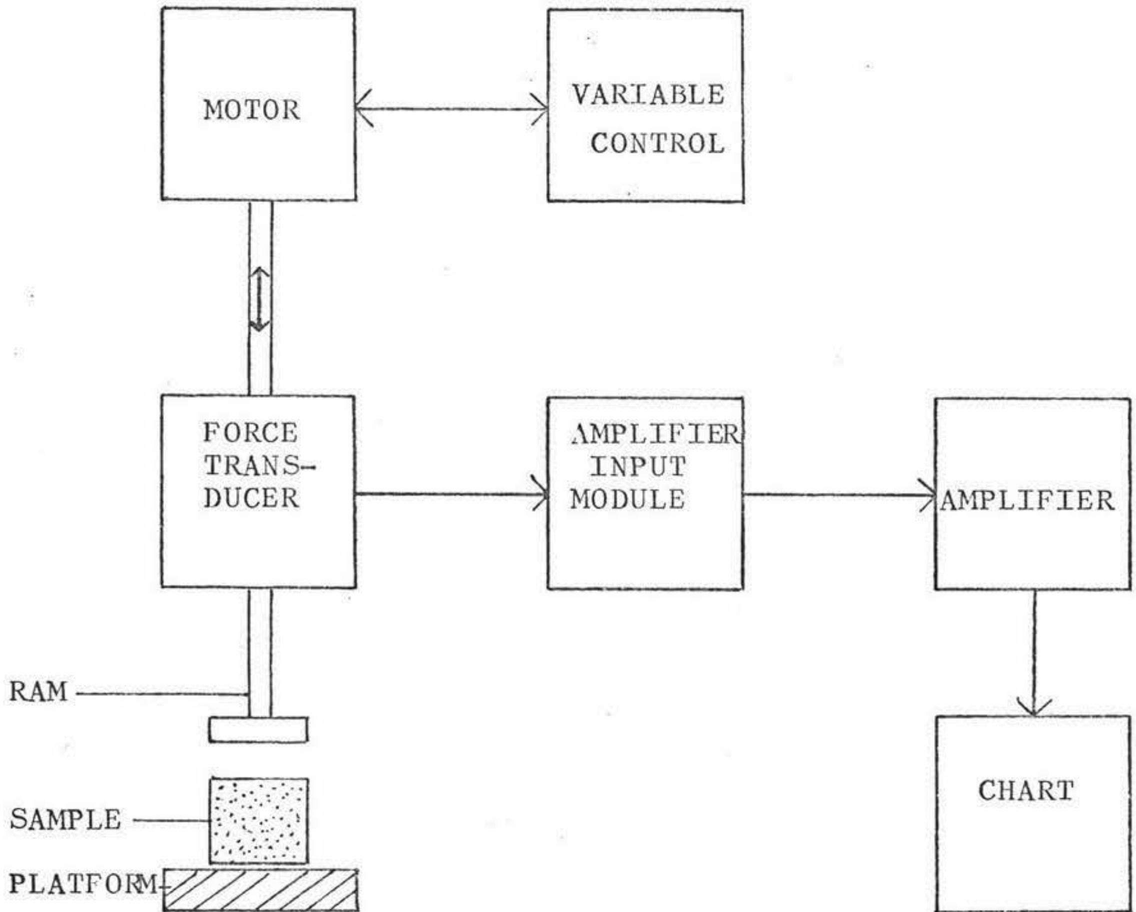
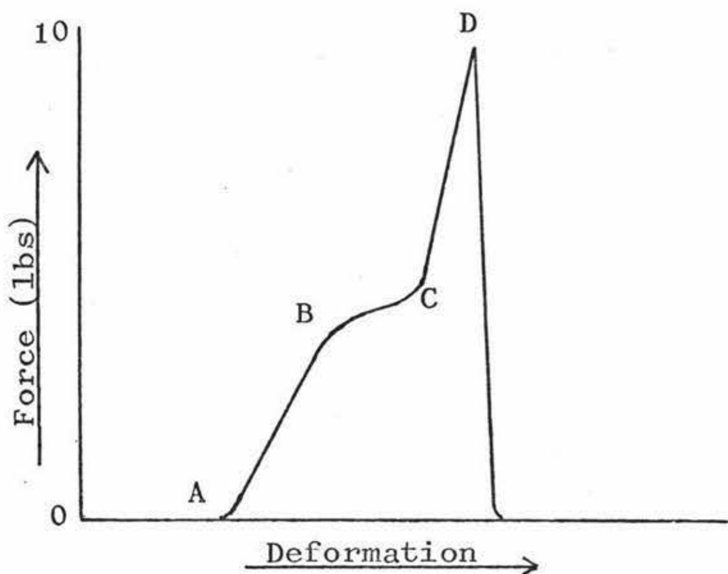


FIGURE 3:3 Typical force versus deformation curve for a sample of restructured fish



to a strip chart recorder.

The instrument used in this work was fitted with a Strainsert (454 Kg) model FLIU-2SG load cell, with a specified accuracy of $\pm$ 0.25%. Due to the effects of the amplifier and the recorder, the final reading is specified at $\pm$ 0.38% accuracy.

Tests were carried out with the sample placed on a solid base. Force was applied using a 20 cm^2 ram installed in the load cell. The operation of the texture measuring instrument used under these conditions is illustrated in Figure 3:2.

Instrument Setting

The variable speed control was set at zero, giving a ram speed of 0.725 mm/sec. The instrument was run on single cycle control, causing the ram to return automatically after it had reached the limit of its stroke, 20 mm above the platform.

The amplifier sensitivity control was set at 20% and the recorder was operated at an input level of 5 mv. This gave a full scale deflection across the chart for 10 lb force.

The chart speed was set at 5 sec/inch (0.2 inch/sec).

Output on Chart Recorder

When samples of restructured fish were tested on the OTMS, with the settings adjusted as outlined above, the resultant curves obtained on the recorder were all variations of the typical force versus deformation curve shown in Figure 3:3.

The force required to deform a sample can be read directly from the vertical axis, which is divided into ten segments on the chart paper. The instrument settings used thus indicated an incremental force of 1 lb per segment.

The horizontal axis was calibrated in terms of deformation by the use of a multiplier matching the chart speed to the ram speed:

$$\text{Percent deformation} = \frac{1 \times \text{ram speed} \times 100}{\text{height of sample} \times \text{chart speed}} \%$$

where 1 is the length along the chart in inches (1)

The chart speed, ram speed and the height of the sample were all kept constant throughout the experiment. Substituting for these constant values equation (1) becomes:

$$\text{Percent deformation} = 1 \times \frac{0.725 \times 100}{0.5 \times 5.08}$$

$$= 1 \times 28.5\% \quad (2)$$

Interpretation of Results

The force versus deformation curve (Fig. 3:3) represents the deformation produced by applied forces. The deformation of the sample passes through its elastic phase (A-B) to its yield point at B. The region in which the sample is rupturing is due to the effect of shearing forces occurs between B and C. Beyond C, where the curve begins its steep upward slope, there is increasing resistance to the compression of the fragmented sample, the force applied causing it to spread over a widening area. This latter portion of the curve was found to be highly variable, and data from it was not used. This variability was considered to arise from the random nature of the fragmentation.

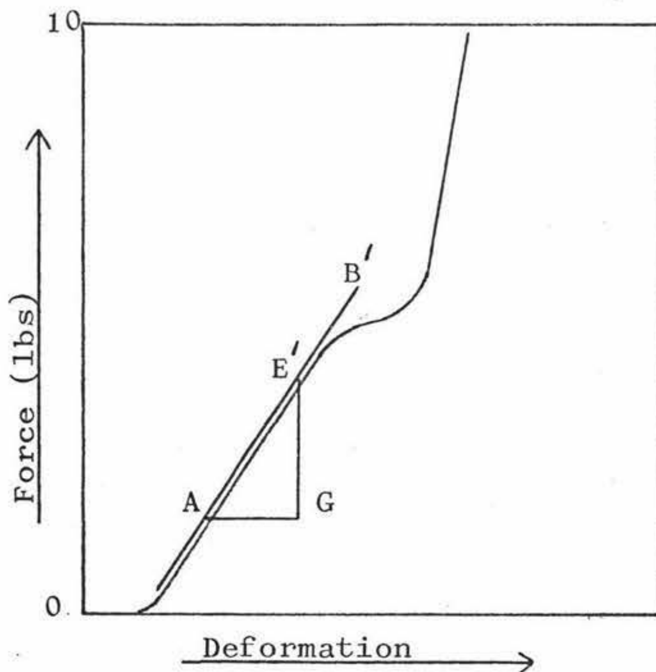
Sections A-B and B-C of the upward sloping curve were evaluated in terms of three variables, Youngs Modulus (resilience), Average Break Force (strength) and the Degree of Breakage (crumbliness) of the product.

1. Youngs Modulus of the Product Prior to Rupture

The ratio of the tensile stress to tensile strain is known as Youngs Modulus, and is a constant for stresses less than the elastic limit of the material. In this work Youngs Modulus was used as an index of the resilience of the product during the initial compression stage.

The ratio was evaluated directly from the curve, by measuring the slope of the straight portion of the curve. This was done by projecting this straight portion as a line AB' (Figure 3:4). A line AG was then drawn along the horizontal axis 1 in. long, and a perpendicular line from G was drawn to cut AB' at E'. The value for E' could be read directly from the chart in pounds weight, and was the force required to give a displacement of 28% (from Equation (2)).

FIGURE 3:4 Evaluation of Youngs Modulus



Having found E' Youngs Modulus can be calculated in standard units as follows:

By definition

$$\text{Youngs Modulus} = \frac{\text{stress}}{\text{strain}}$$

$$= \frac{\text{Force}}{\text{Area of sample}} \div \text{Deformation} \qquad (3)$$

The point E' is defined as the force required to give a 28.5% deformation (Figure 3:4); when the area of the sample is 0.384 in.^{2} (0.7 in. diameter).

By substituting these values in Equation (3)

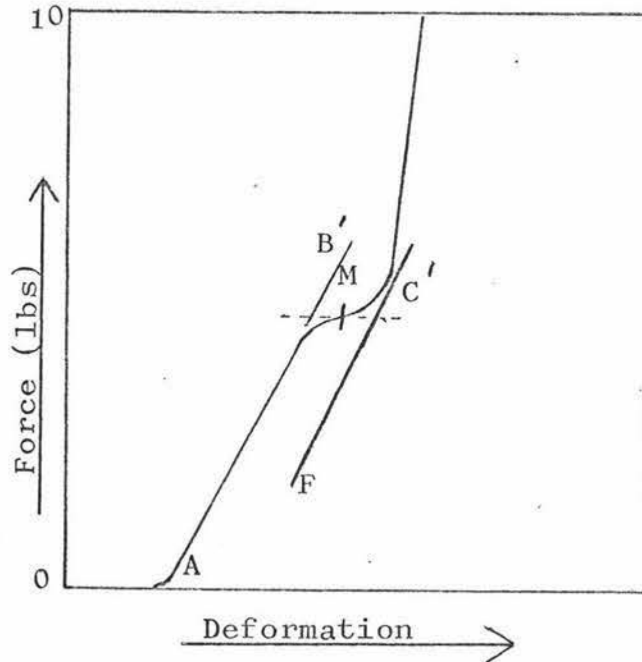
$$\text{Youngs Modulus} = E = E' \times 0.135 \text{ lb/in.}^2$$

2. Average Break Force

The yield point, or the point at which the sample commences to rupture, was found to be very difficult to measure or define on a typical force versus deformation curve (Figure 3:3). For this reason an Average Break Force was used instead.

The Average Break Force was taken as the point of that portion of the curve during which rupture of the sample occurs; that is, B-C. This was most readily done by drawing a line C-F parallel to AB' at a tangent to the point C (Figure 3:5). The mid point of the curve representing the rupture phase (M), was determined, and the force at this point taken directly from the chart in pounds force.

FIGURE 3:5 Evaluation of the Average Break Force



3. Degree of Breakage

The crumbliness or the Degree of Breakage of the product is an important textural characteristic which was found to vary with different treatments. The degree of crumbliness was found to be related to the deformation which occurred from the yield point to the point where the curve begins its steep upward slope. This deformation can be measured in inches directly from the chart, as shown in Figure 3:6, by measuring the distance between the perpendicular projection from the base to the inflections on the curve.

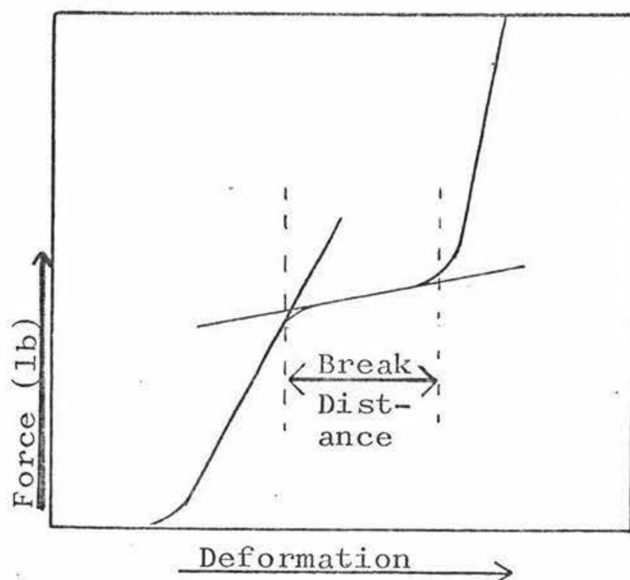
The Degree of Breakage can be calculated as a percentage as follows:

$$\text{Degree of breakage} = \frac{\text{Distance ram moved during rupture}}{\text{Sample height}}$$

From Equation (2)

Degree of breakage = Break distance x 28.5%

FIGURE 3:6 Evaluation of the break distance



EXPERIMENTAL WORK

Experiment 1

Textural Characteristics of Cooked Fish

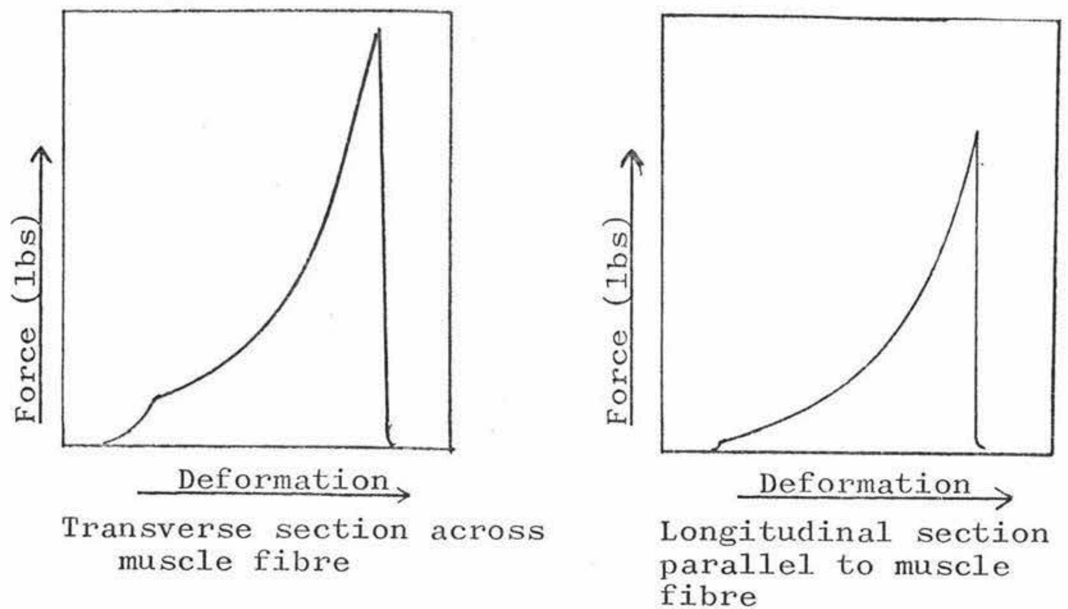
Trevally fillets were placed in a pyrex steamer and steamed for 20 minutes. Samples of the fillet were taken after it had cooled and then stored for 24 hours in the chiller at $0^{o}C$. Test plugs of the fish were taken with a No. 12 cork borer. The samples were cut to a height of 0.5 inches, giving a cylinder 0.7 inches in diameter and 0.5 inches high.

The plugs of fish were tested on the OTMS in the usual manner, a typical curve obtained is shown in Figure 3:7.

These curves indicate that the textural characteristics of cooked fish are slightly different to those of the restructured sample (Figure 3:3). Very low forces are required to bring about the initial rupture of the test sample, the value being slightly higher across the sample than along the sample. Deformation of the sample causes the fibres to break apart

from one another, and be spread over the surface of the platform. The increasing resistance of the product to further deformation is in part due to the spreading of the product over a wide area, but also indicates that although the cooked fish is readily broken into fibres, the fibres themselves are not easily broken down as compacted by compression.

FIGURE 3:7 Typical force versus deformation curve for steamed trevally fillet



Although the textural characteristics of the fish did not conform to a force versus deformation pattern typically found for the restructured product, values for the three variables outlined above could still be determined. The average values of three samples are given in Table 3:1, and serve as a guide as to what is required in the restructured product.

TABLE 3:1 Textural Characteristics of Cooked Trevally Fillets in Terms of Youngs Modulus, Average Break Force and The Degree of Breakage

Classification	Mean Value
Youngs Modulus	0.3 lb/in.^{2}
Average Break Force	0.5 lb
Degree of Breakage	30.0 %

Experiment 2

Comparison between the binding characteristics of trevally and gurnard

Note The comparison between the binding characteristics of trevally and gurnard was actually done well towards the end of the experimental work when it became apparent that these fish would have to be used as alternatives in the study if the work was to be completed on time. The results of the comparison are reported here to avoid confusion which may arise due to different species of fish being used when the thesis is read.

Restructured samples of trevally and gurnard mince were prepared without additives as described above. The average values of three tests from each sample is given in Table 3:2.

TABLE 3:2 Comparison between Binding Characteristics of Restructured Trevally and Gurnard

Classification	Mean Value	
	Trevally	Gurnard
Youngs Modulus	0.48 lb	0.206 lb/in.^{2}
Average Break Force	2.90 lb	1.43 lb
Degree of Breakage	31.2 %	30.5 %

Experiment 3

Effect of Salt Concentration

Samples were made up, as described earlier, with salt added and mixed into the fish to the extent shown in Table 3:3. The mince used in this experiment was material which had been processed on the meat/bone separator and sent to Massey University from Auckland in a frozen state.

TABLE 3:3 Samples prepared to determine the effect of sodium chloride on the binding characteristics of restructured trevally mince

Sample No.	1	2	3	4	5	6	7	8
Fish mince (g)	150	150	150	150	150	150	150	150
Salt (g)	-	0.15	0.30	0.45	0.60	0.90	1.20	1.50
Salt added (%)	-	0.1	0.2	0.3	0.4	0.6	0.8	1.0

Results and discussion

The results obtained by evaluating the OTMS curves are shown in Figures 3:8 to 3:10, in which the level of salt (vertical axis) is plotted against Youngs Modulus, the Average Break Force and the Degree of Breakage determined as described in the previous section. The range over the three tests made on each sample is indicated by the bars about the mean value recorded on the graphs.

Examination of the results shown in Figures 3:8 to 3:10 suggests that the effects of adding salt varies according to concentration and that there appears to be two fairly distinctly different processes involved.

FIGURE 3:8 Change in Youngs Modulus with increasing Salt Content

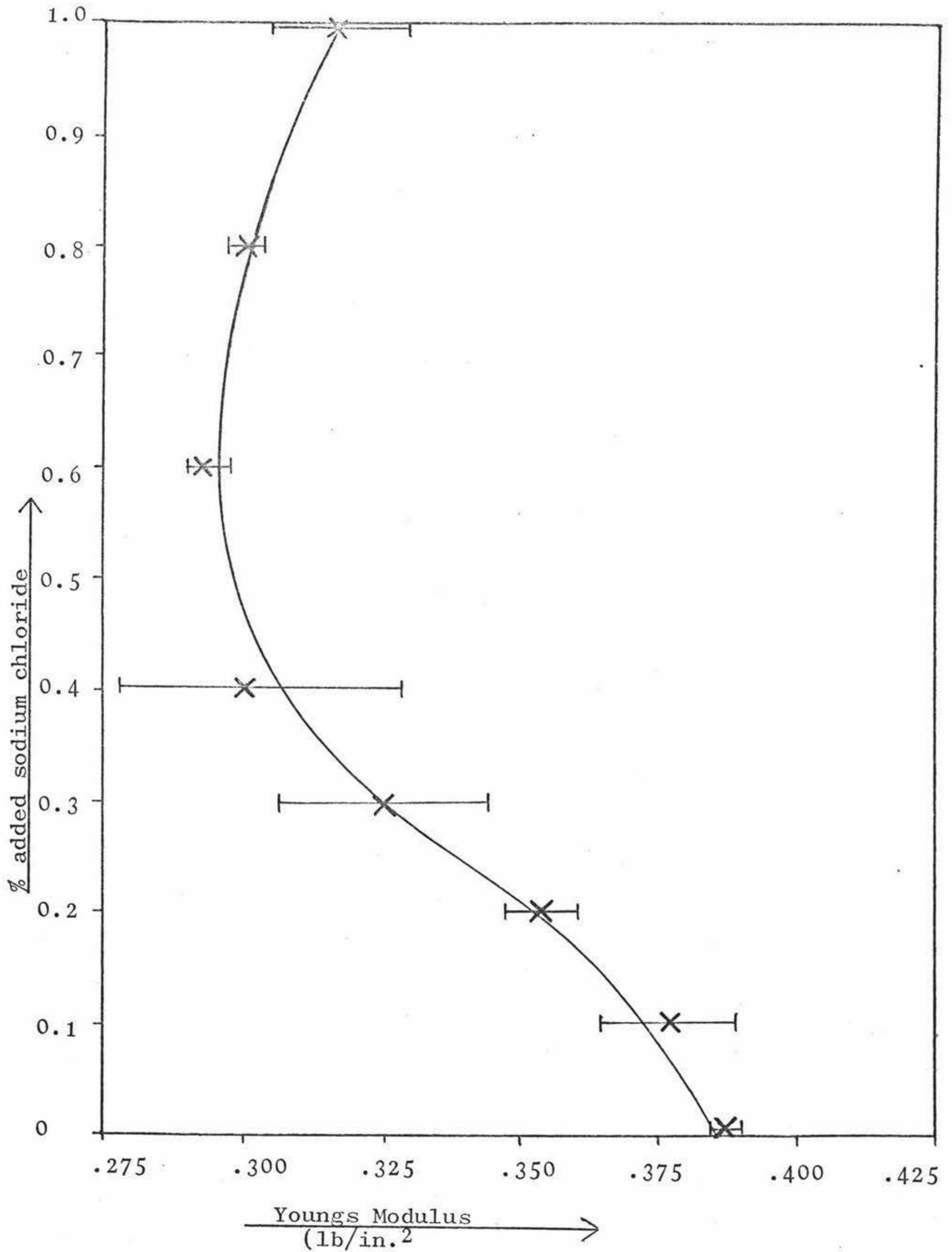


FIGURE 3:9 Change in the Average Break Force with Increasing Salt Content

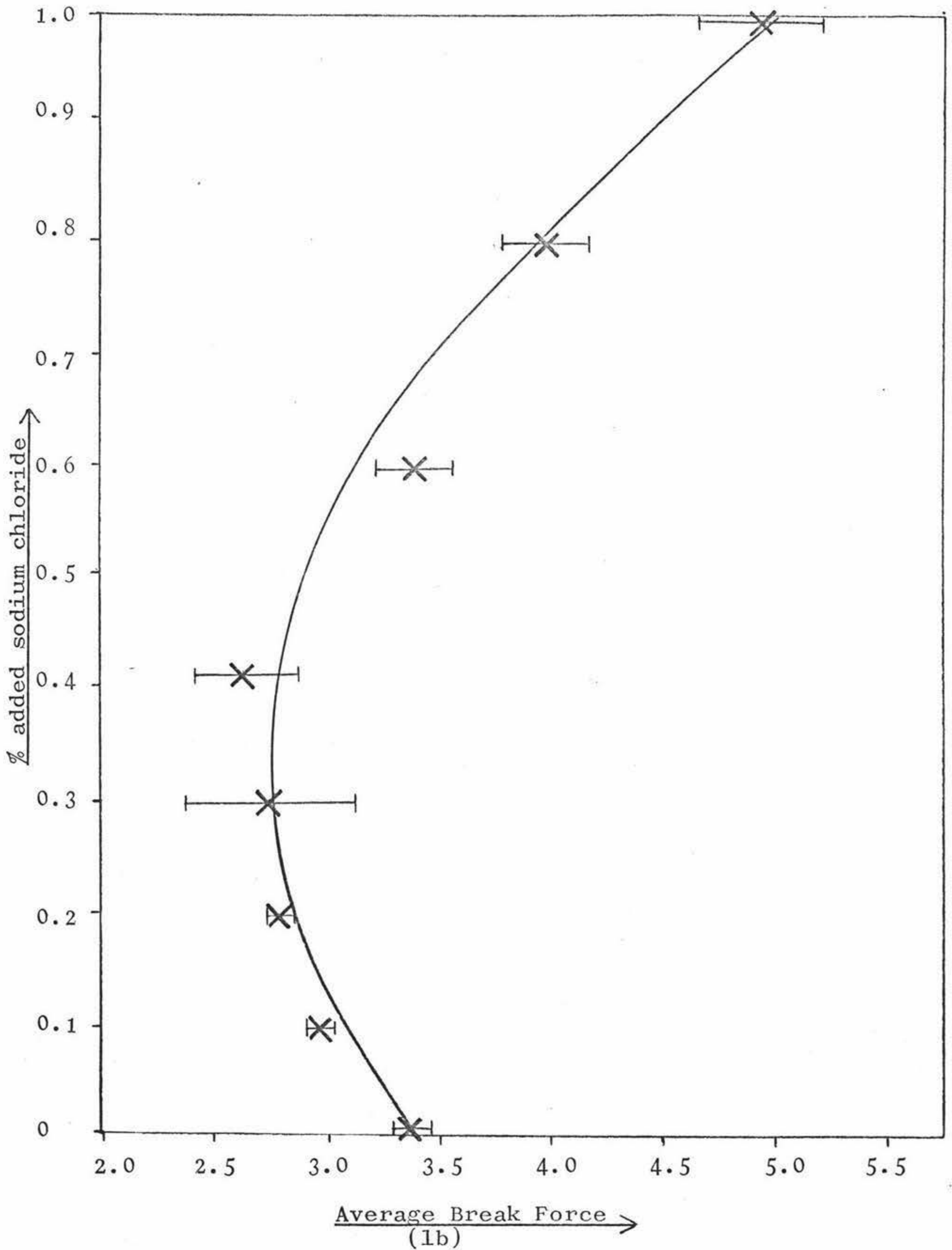
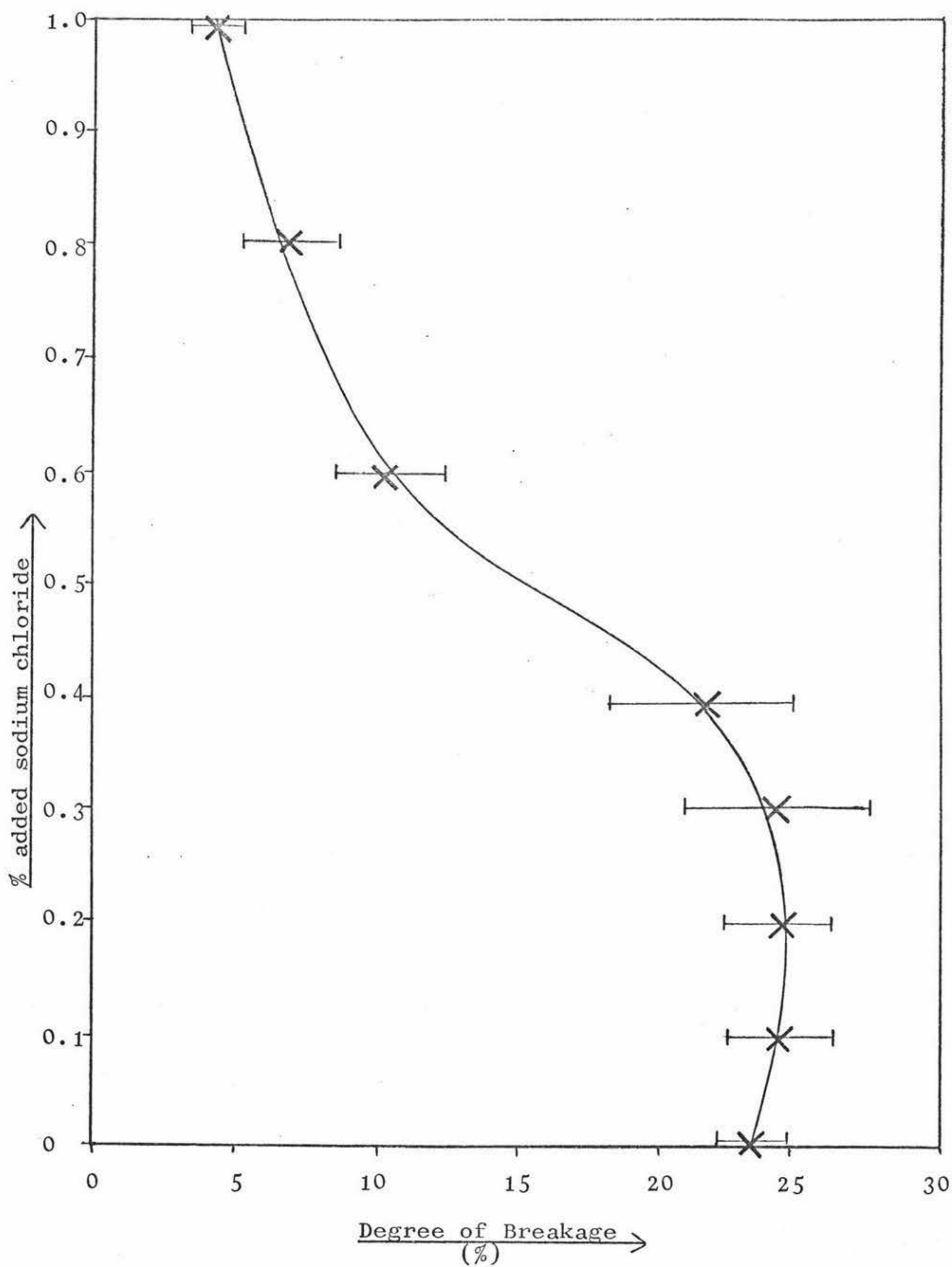


FIGURE 3:10 Change in the Degree of Breakage with increasing Salt Content



As salt is added from 0.0 to 0.4% there is a sharp drop in the resilience (Youngs Modulus) of the material prior to rupture. This effect is probably due to the formation of an increasing quantity of gel, the material forming a gel being the salt soluble myofibrillar protein and moisture drawn from the mince particle. As salt is added to 0.4% there is also a slight drop in the force required to rupture a sample, which is consistent with the concept of an increasing proportion of the gel structure being present and there being less fine fibrous material for reinforcing the gel. There was no change in the crumbliness of the product (Degree of Breakage).

If salt is added beyond 0.4% there is an increase in the binding strength of the restructured product, as is shown by the increase in the Average Break Force and the decrease in the Degree of Breakage. These changes can also be discussed in terms of the eating quality of the product. Below 0.4% salt added, the product tended to have a dry mouth feel. As the added salt was increased to 1%, the product appeared more moist and sticky, simultaneously becoming increasingly rubbery to the point of being unacceptable when salt was added in excess of 0.8%.

The reactions and properties of the myofibrillar proteins have been reviewed in Section II, and indicate that the type of interaction which occurs is governed by the salt level in the medium.

In isolation, myosin is reported to form aggregates. At an ionic strength of less than 0.35M, the aggregates are formed by a reversible process, whereas above 0.5M the formation of the aggregate is irreversible, apparently due to

tertiary structural change (Perry 1967).

The interactions of actin and myosin are also known to be important in binding in heat coagulated meat or fish gels (Fukazawa et al 1961 a, b). At a salt concentration of 0.1 to 0.15M the interaction between actin and myosin results in a fine suspension being formed (Gergely 1970), and at about 0.5M there is a sharp increase in viscosity.

In this work a change in the character of the protein gel was first noticed after about 0.4% salt had been added to the mixture. This change in textural properties can be seen in the drop in the crumbliness (Degree of Breakage, Figure 3:10) and the increase in binding strength (Average Break Force, Figure 3:9) which occurs when salt is added in excess of 0.4%.

The initial level of salt in flesh is about 0.15M (Gergely 1970). An addition of 0.4% salt would bring this level to about 0.25M. This level is about half the value that might have been expected to produce the observed changes based on the work reported in purified systems of myofibrillar proteins. It may be that a portion of the myofibrillar protein can react to form the types of aggregates which form highly viscous sols which give strong gels upon heating when as little as 0.25M salt is present. Thus the increase in the binding strength when the salt is added in excess of 0.4% could be explained as a direct influence of the concentration of the salt.

Alternatively, it may be that in the composite system dealt with here, the effect of cellular structures, osmotic forces and chemical interactions is such that only a portion of the water in the system is available as a medium for the

interaction of the myofibrillar proteins. This suggestion could also help to explain the higher than predicted osmotic pressure which results when salt is added to muscle tissue, (Lawrie 1966), and so in effect the reactions considered will be occurring at molar concentrations much nearer those found to effect these changes in the simpler systems. This latter appears the more likely explanation.

Experiment 4

Effect of adding salt and water to the mixture prior to cooking

A. Effect of varying the salt content at different moisture levels

Two series of samples were prepared, the first with 7.5 ml (5%) added water, and the second with 15 ml (10%) added water. The other ingredients were added as shown in Table 3:3.

Results and Discussion

Results evaluated from the OTMS curves are shown in Figures 3:11 to 3:13.

The curves based on the results from this experiment follow a similar trend to the curves shown in the previous experiment (Figure 3:8 to 3:10). By comparing the values from Figures 3:8 to 3:10 with Figure 3:11 to 3:13, it can be seen that the addition of water results in the product becoming less resilient, breaking up under lower stress and crumbling more readily than do the samples with equivalent salt contents in which no water is added.

The apparent change in the nature of the gel formed, resulting in an increase in the Average Break Force and a

FIGURE 3:11 Change in Youngs Modulus with Increasing Salt Content where 5% and 10% water have been added

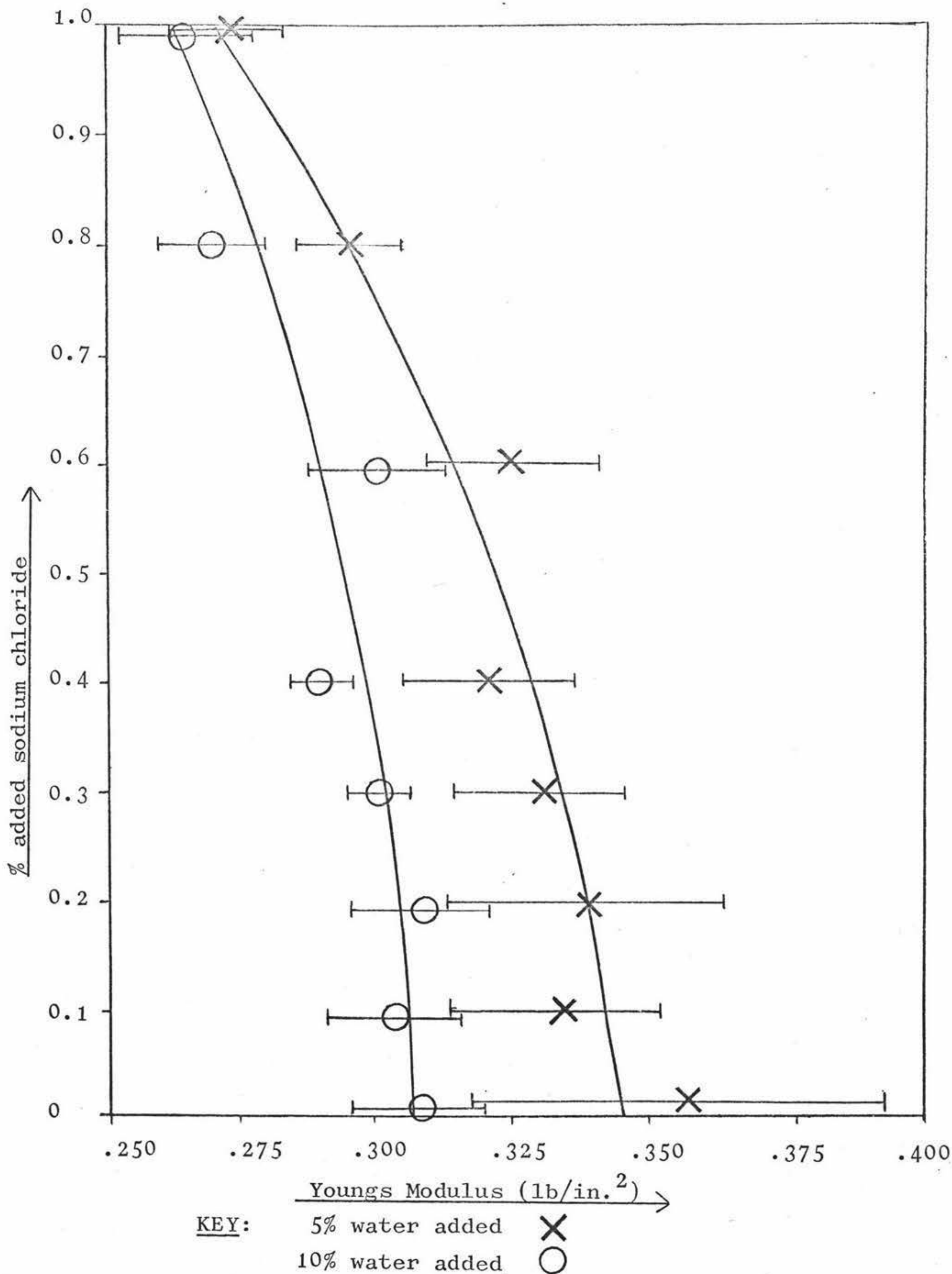


FIGURE 3:12 Change in the Average Break Force with Increasing Salt Content where 5% and 10% water have been added

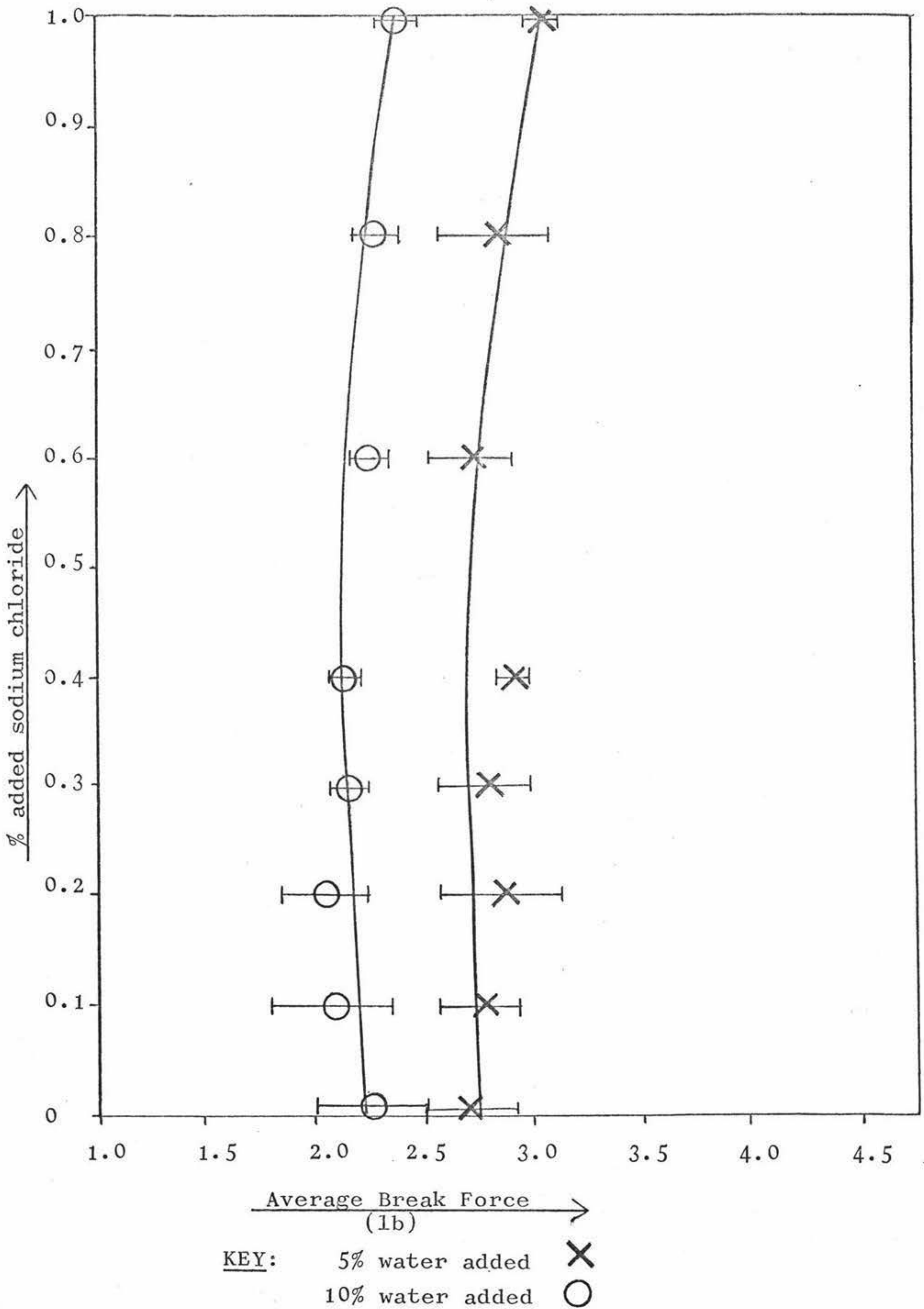
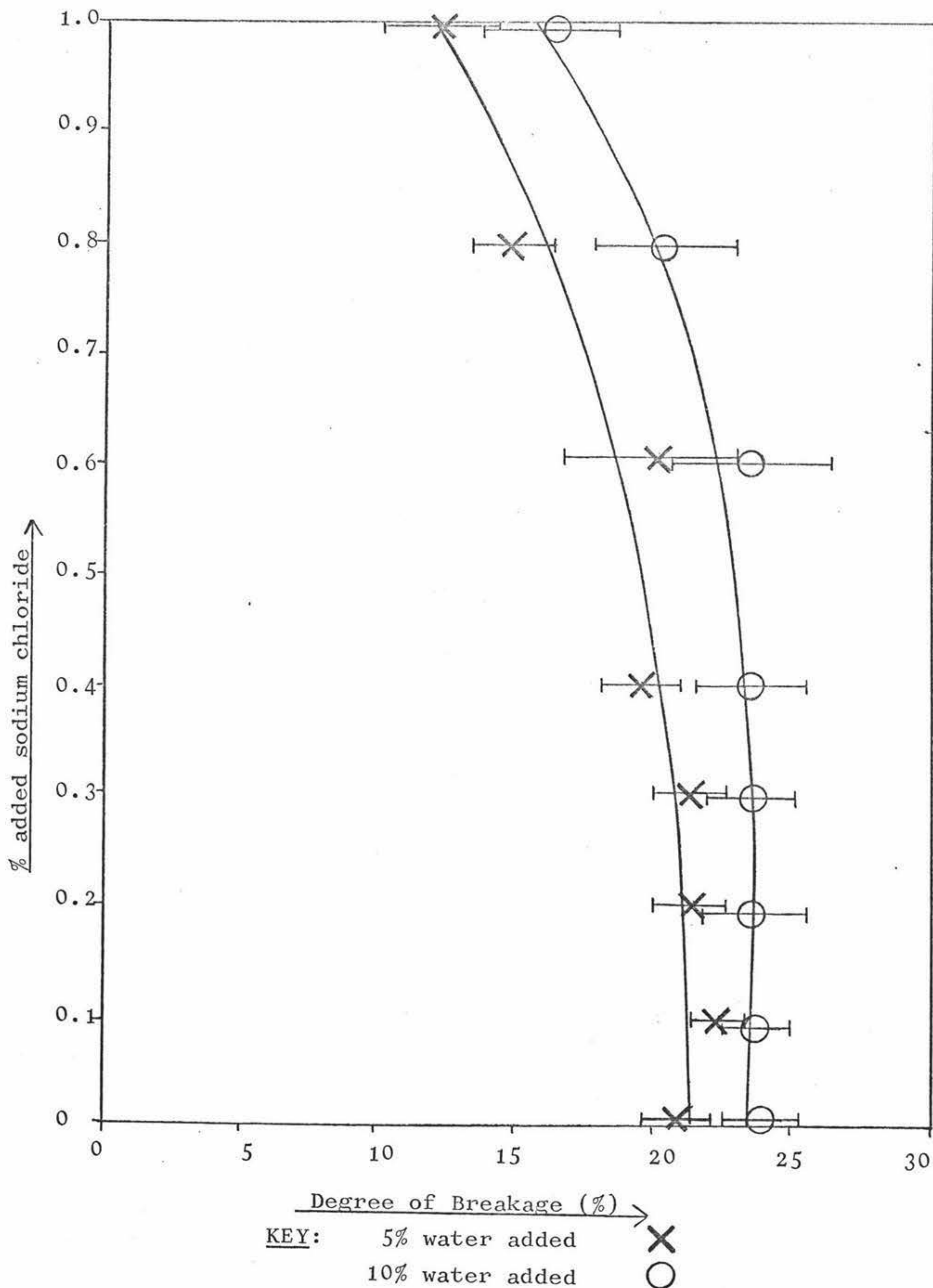


FIGURE 3:13 Change in the Degree of Breakage with Increasing Salt Content where 5% and 10% Water have been added



decrease in the Degree of Breakage was discussed in the last experiment. The discussion applies to the results reported in Figures 3:11 to 3:13. In these results it can be seen that the change in character does not occur until 0.6% to 0.8% salt has been added to the sample, the higher salt requirement being a reflection probably of the effect of the added water on the affective molar concentration as discussed in the previous section.

B. Effect of the moisture content, keeping the salt level constant

Samples were prepared to contain 1% added salt, water being added as shown in Table 3:4. The fish used in this experiment was gurnard which had been processed and frozen in Auckland.

TABLE 3:4 Samples prepared to find the effect of adding water to the gelled fish product

Sample No.	1	2	3	4	5	6	7	8
Fish Mince (g)	150	150	150	150	150	150	150	150
Salt (g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Water (ml)	0	3	6	9	12	15	18	21

Results and Discussion

The effect of adding water to the mixture was evaluated by the OTMS, and the results are shown in Figures 3:14 to 3:16.

It can be seen that the resilience (Youngs Modulus) and the binding strength (Average Break Force) both decrease with the addition of water. This would be expected as the

FIGURE 3:14 Change in Youngs Modulus with Increasing the Moisture Content while Maintaining the Salt Content at 1%

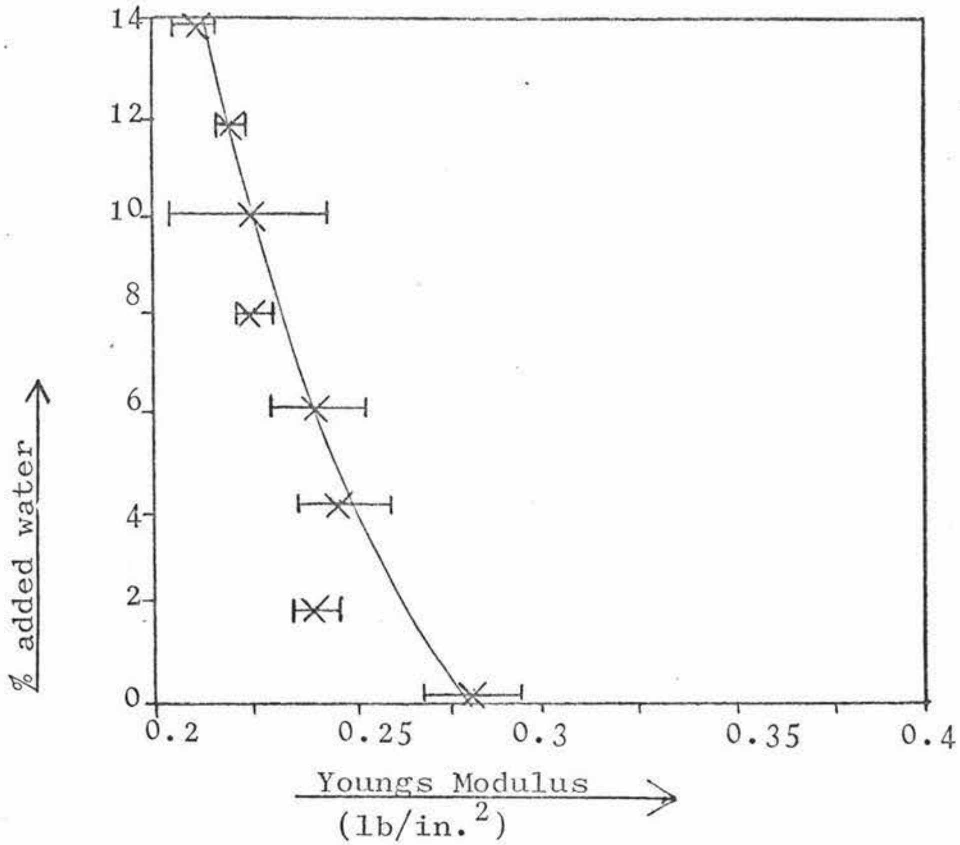


FIGURE 3:15 Change in the Average Break Force with Increasing Moisture Content while Maintaining the Salt Content at 1%

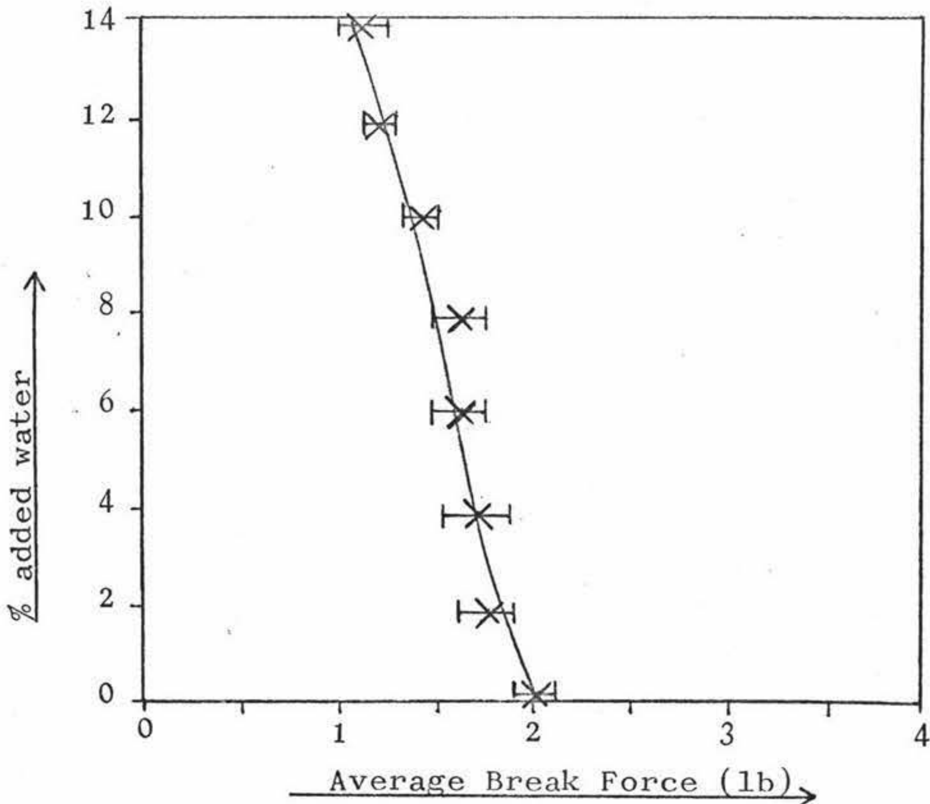


FIGURE 3:16 Change in the Degree of Breakage with Increasing Moisture Content while Maintaining the Salt Content at 1%

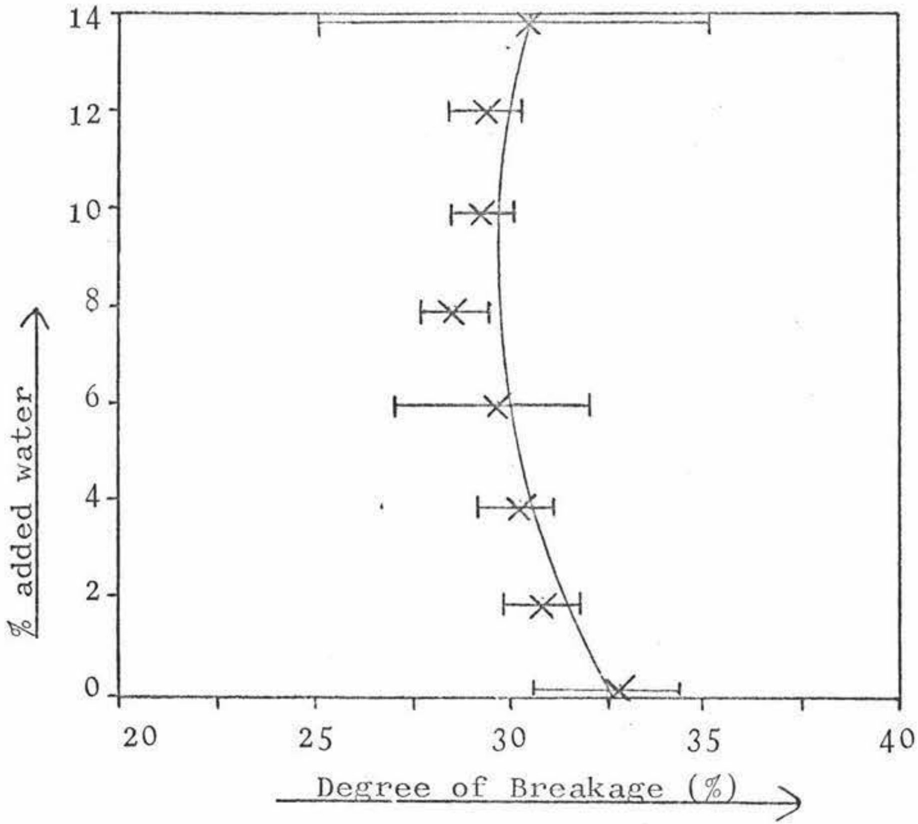
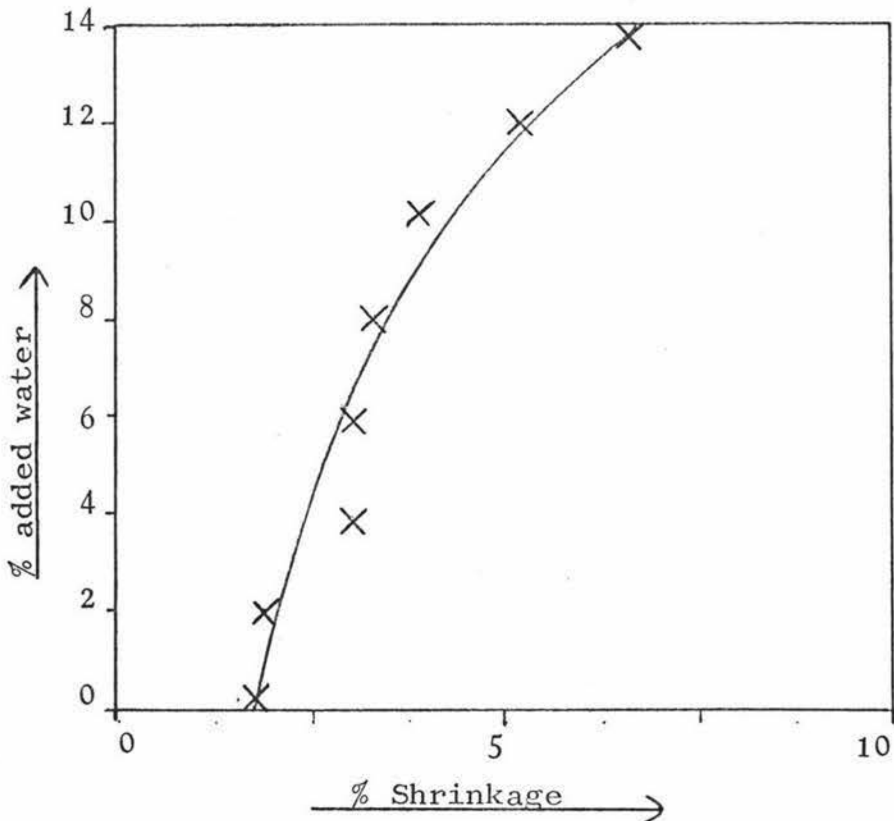


FIGURE 3:17 Effect of the Addition of Water on the Shrinkage of Restructured Gurnard Mince



addition of water would dilute dissolved proteins. The Degree of Breakage on the other hand does not appear to be affected.

The addition of water was also noted to cause shrinkage of the product on cooking as shown in Figure 3:17. The increased shrinkage which occurs as more water is added is probably due to the product having a limited water holding capacity. The loss in volume upon cooking would be related to the amount of moisture released as a result of the heat denaturation of the protein.

Experiment 5

Effect of the addition of phosphate

In view of the work published, it was expected that the addition of phosphates would affect both the binding characteristics (Nakayama and Sato 1972, Fukazawa et al 1961c, Vadehra and Baker 1970), and the water holding capacity (Bendall 1954, Lawrie 1966) in the cooked product.

Preliminary experiments were carried out on frozen trevally, which was thawed, then skinned and boned. The cleaned fish was passed through a mincer fitted with a 5 mm plate. Both sodium hexametaphosphate and tetrapotassium pyrophosphate were tried in these experiments. Levels of phosphate tested were 0.025%, 0.05%, 0.1% and 0.25%. The tests were carried out on samples with varying salt contents as outlined in Table 3:3.

There was no observed variation between the results of the samples to which phosphates had been added and the results of the controls; Figures 3:8 to 3:10. Shrinkage varied from about 3% where no salt was added to no shrinkage

when more than 0.8% salt was added, but there was no change in shrinkage due to the addition of the phosphates at the levels used in these experiments.

Fukazawa et al (1961c) reported that the effect of phosphates was dependent on the nature of the protein, and that only pyrophosphates have activity to split actomyosin, a property that was maintained even though the protein was denatured. Other phosphates, such as sodium hexametaphosphate, do not split actomyosin, and if the protein is denatured the effect of the phosphate was found to be due only to an increased extraction of light meromyosin. Although light meromyosin does not affect the binding character of heat gelled systems, it should affect the water holding capacity and hence the shrinkage of the product.

The fish used in this experiment, which was repeated four times, had not been specially selected and had been stored frozen, and so may have undergone some changes affecting muscle properties. Its use was justified on the basis that it represents typical raw material for the process being investigated.

In view of the above results, the four sets of experiments were repeated using fish which had been specially selected for their quality, and had then been processed, frozen, and air freighted to Massey University. The samples examined were based on the mixtures shown in Table 3:5.

TABLE 3:5 Mixtures prepared for the evaluation of phosphates from freshly processed fish

	Sample No.		1	2	3	4	5
Experiment I	Fish TPP	(g) (g)	150 0.15	150 0.30	150 0.45	150 0.60	150 0.75
Experiment II	Fish Salt TPP	(g) (g) (g)	150 1.5 0.15	150 1.5 0.30	150 1.5 0.45	150 1.5 0.60	150 1.5 0.75
Experiment III	Fish HMP	(g) (g)	150 0.15	150 0.30	150 0.45	150 0.60	150 0.75
Experiment IV	Fish Salt HMP	(g) (g) (g)	150 1.5 0.15	150 1.5 0.30	150 1.5 0.45	150 1.5 0.60	150 1.5 0.75

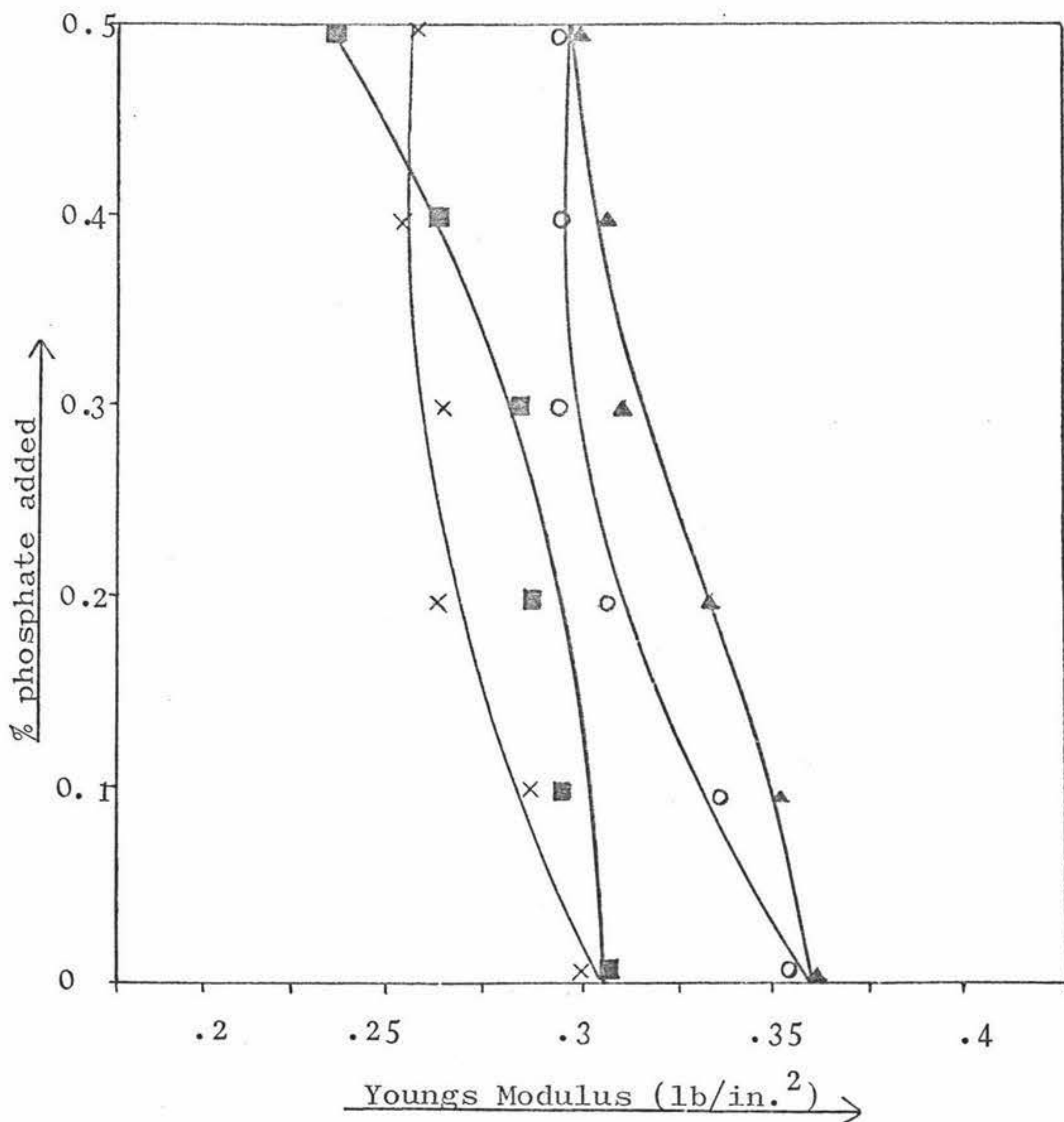
Results and Discussion

The results of the effect of phosphates on the binding characteristics of the heat gelled product is shown in Figures 3:18 to 3:20.

Effect of adding phosphates without the addition of salt

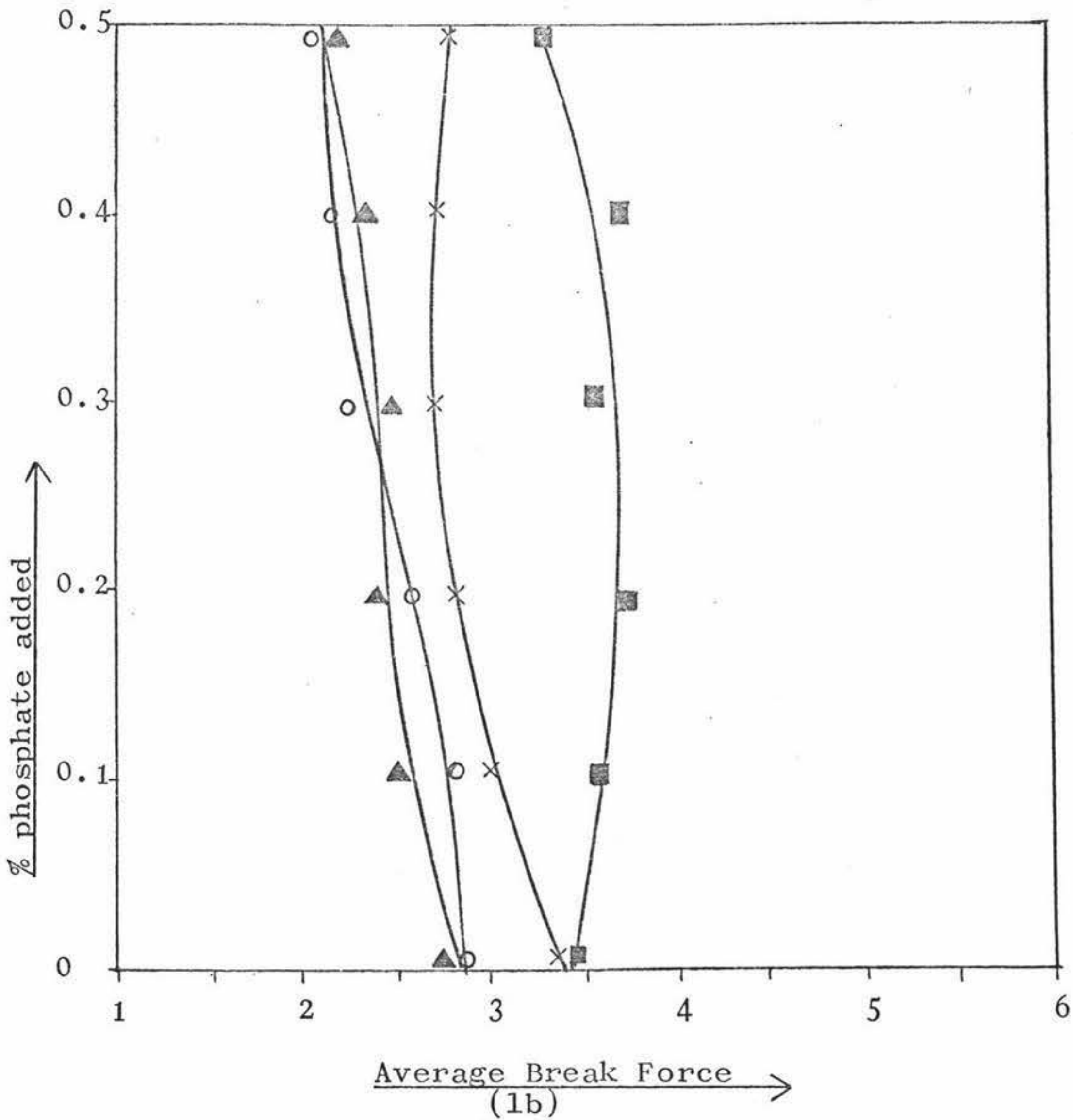
The addition of both sodium hexametaphosphate and tetrapotassium pyrophosphate to the fish mince resulted in a gelled product being formed having slightly weaker binding characteristics than when no phosphates had been added. This can be seen in the slight drop in the values of the Average Break Force and the resilience of the product. Simultaneously there is a slight increase in the tendency of the product to crumble under stress, as can be seen by the slight increase in the values for the Degree of Breakage.

FIGURE 3:18 Effect of Phosphate on the resilience (Youngs Modulus) of heat gelled fish mince



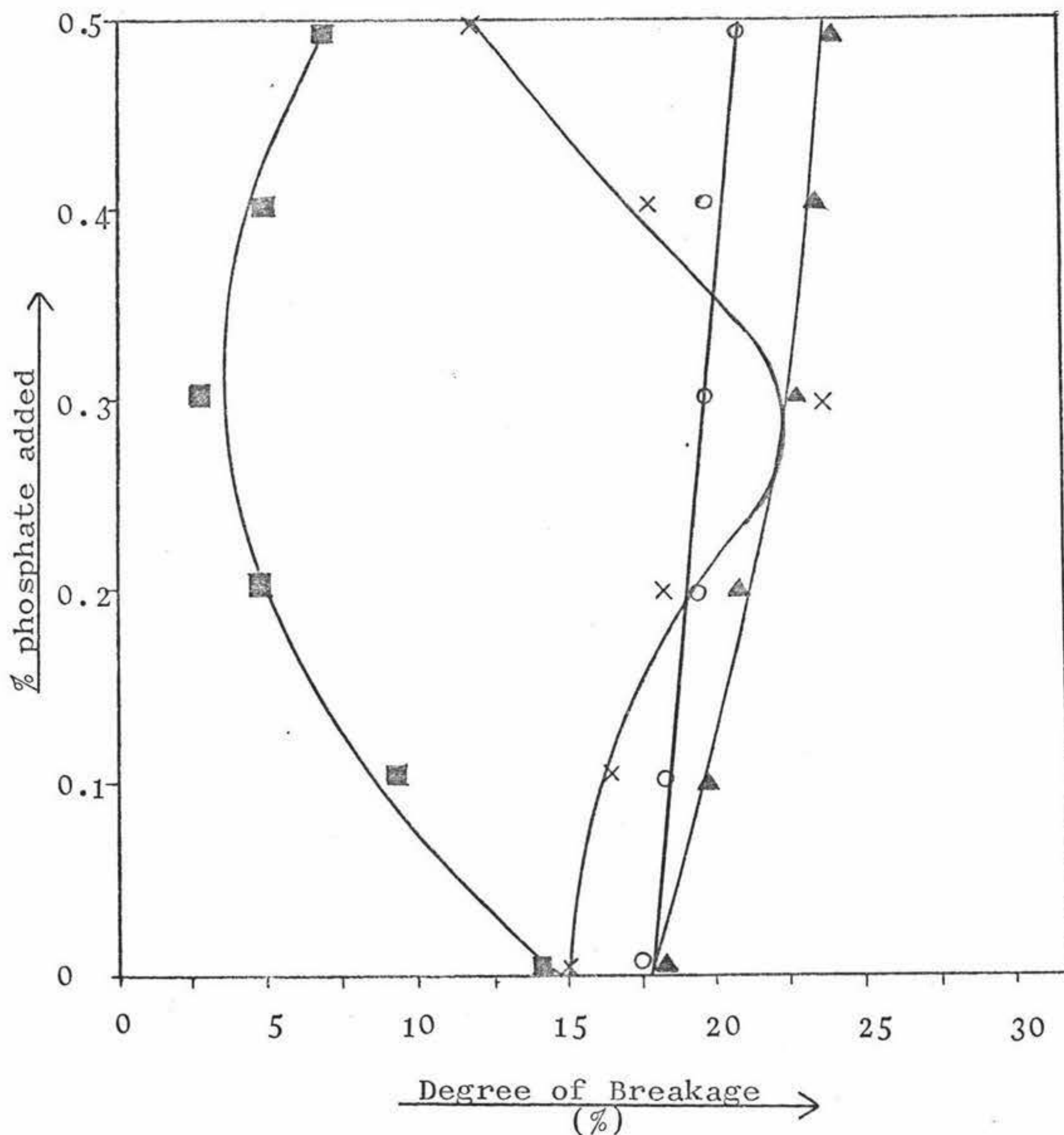
KEY:
- ○ Sodium Hexametaphosphate
- ▲ Tetrapotassiumpyrophosphate
- × Sodium Hexametaphosphate in the presence of 1% added salt
- ■ Tetrapotassium pyrophosphate in the presence of 1% added salt

FIGURE 3:19 Effect of phosphates on the binding strength (Average Break Force) of heat gelled fish mince



KEY:
- o Sodium Hexametaphosphate
- ▲ Tetrapotassiumpyrophosphate
- × Sodium Hexametaphosphate in the presence of 1% added salt
- ■ Tetrapotassium pyrophosphate in the presence of 1% added salt

FIGURE 3:20 Effect of phosphates on the crumbliness (Degree of Breakage) of heat gelled fish mince



KEY:
- o Sodium Hexametaphosphate
- ▲ Tetrapotassium-pyrophosphate
- × Sodium Hexametaphosphate in the presence of 1% added salt
- ■ Tetrapotassium pyrophosphate in the presence of 1% added salt

The slight weakening effect which occurred under these conditions could arise due to the effect of the phosphates drawing more moisture into the external phase of the mixture, thereby having an effect of diluting the protein forming gel structures upon heating. The phosphates may have assisted in solubilising some of the myofibrillar protein, but at a low molar concentration which occurs in the absence of added salt, this may not have had any effect on the binding characteristics of the gel formed upon heating.

The shrinkage of the product in which no salt was added was about 5%, with no apparent effect due to the addition of phosphates.

Effect of phosphates in the presence of 1% added salt

In this experiment some of the curves from the OTMS were difficult to read due to a very low Degree of Breakage, and variations within the samples were greater than experienced elsewhere.

An explanation for this may be that the equilibration of 24 hours prior to cooking was not long enough for the phosphate and salt to achieve their optimum effect. This possibility was not examined experimentally.

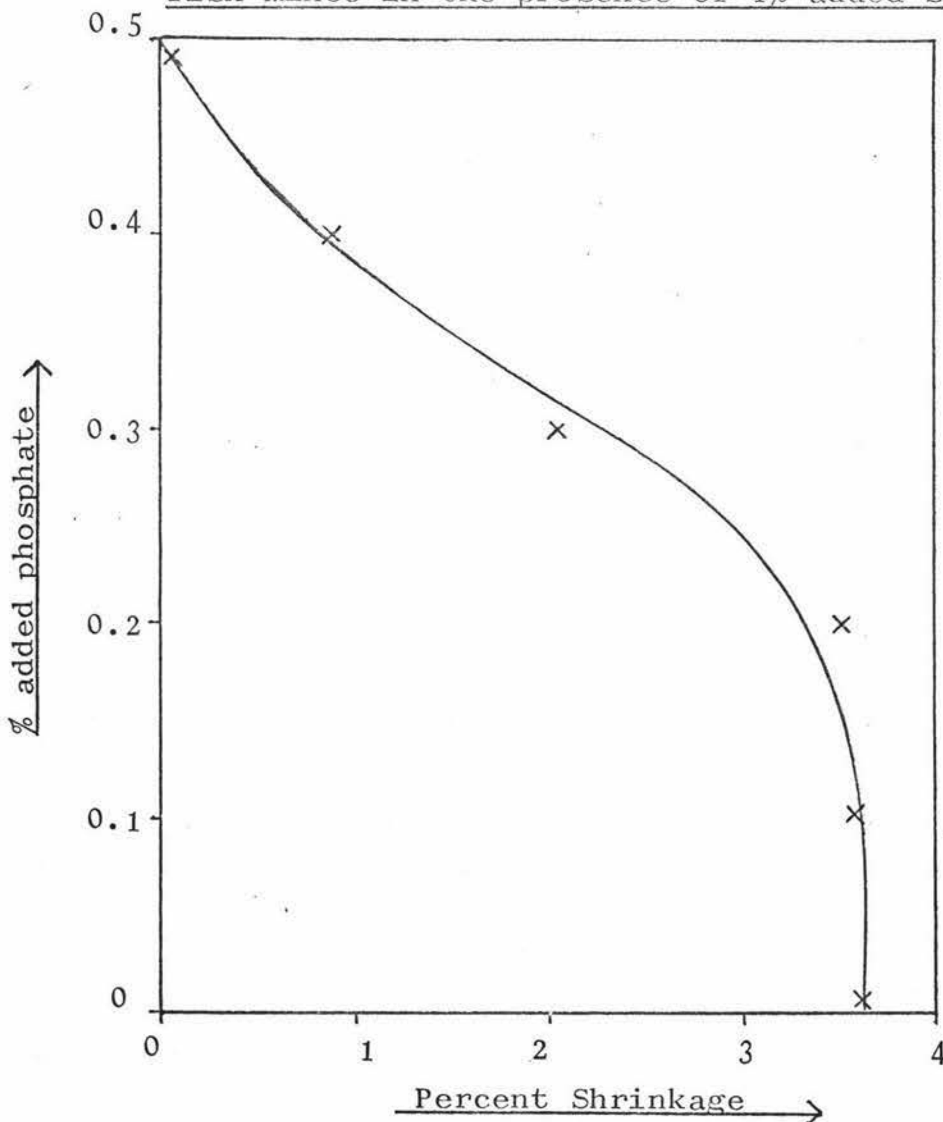
1. Effect of Tetrapotassium pyrophosphate (TPP)

With an addition of up to about 0.3% TPP there appears to be a slight increase of binding in the system. This can be seen as an increase in the Average Break Force and the simultaneous drop in the Degree of Breakage which occurs in this region. Further increases of TPP to 0.5% do not appear to continue the strengthening effect, and may in fact have caused

a slight drop in binding as indicated in the increase in the Degree of Breakage.

In this experiment the addition of TPP to the fish mince in excess of 0.3% did have a measurable effect in reducing shrinkage on cooking as shown in Figure 3:21. These results indicate that the effect of TPP increases with concentration in the fish, and causes increasing binding strength up to a level of 0.3%, and especially between 0.3 - 0.5% causes an increased water holding capacity as is shown by the decrease in shrinkage.

FIGURE 3:21 Effect of the addition of tetrapotassium pyrophosphate on shrinkage of heat gelled fish mince in the presence of 1% added salt



2. Effect of Sodium Hexametaphosphate (HMP)

Sodium Hexametaphosphate is reported to solubilize actomyosin, but not split the actin-myosin complex. At levels of less than 0.3%, HMP added to the fish in the presence of 1% added salt appears to have had a negative effect on the binding character as can be seen by the slight drop in the Average Break Force, and more dramatically by the increase in the Degree of Breakage to a maximum of 25%, but above 0.3% addition, there was an increase in the binding of the heat gelled product.

The apparent requirement of at least 0.3% HMP before a positive effect occurred and the negative effect of the HMP up to this level is not understood. In view of the dispersion of the results more work is required in this area to confirm this trend but the trends observed were not considered to be important industrially.

There was no change in shrinkage from 3% throughout the experiment, and HMP being neutral did not change the pH of the fish from 6.7 so this could not have been a contributory factor.

Experiment 6

Effect of particle size on the binding characteristics of restructured fish

Gurnard mince which had been processed through a meat/bone separator was used in this experiment. This mince was further fragmented by processing through a meat grinder with variously sized plates. The size of the particles was estimated by separating out individual particles of mince and counting out the particles required for 50 g of product. The

average weight of the particles was calculated as the fraction of 50 g divided by the number of particles.

The range in particle size was obtained by grinding the mince as shown in Table 3:6. The samples were prepared with the addition of 0.4% added salt.

TABLE 3:6 Preparation of fish mince with varying particle size

Sample No.	Preparation	Average Particle Weight (g)
1	Headed and gutted gurnard ex meat/bone separator	0.102
2	Mince ex 1 1x through .390 in. plate	0.029
3	Mince ex 1 2x through .390 in. plate	0.018
4	Mince ex 3 1x through .234 in. plate	0.010
5	Mince ex 3 2x through .234 in. plate	0.008

Results and Discussion

The effect of changing the particle size of the fish mince used in the restructured product is shown in Figures 3:22 to 3:24.

It can be seen that the general effect of reduction in particle size is to increase the chewiness (resilience) and to strengthen the binding characteristics of the gelled product. This becomes more apparent once the average weight of the particle is reduced to below 0.029 g. At this stage there is a rapid increase in Youngs Modulus, which is associated with an unpleasant rubbery texture. The Average Break Force also increases in this region.

FIGURE 3:22 The Effect of particle size on the resilience of restructured fish mince

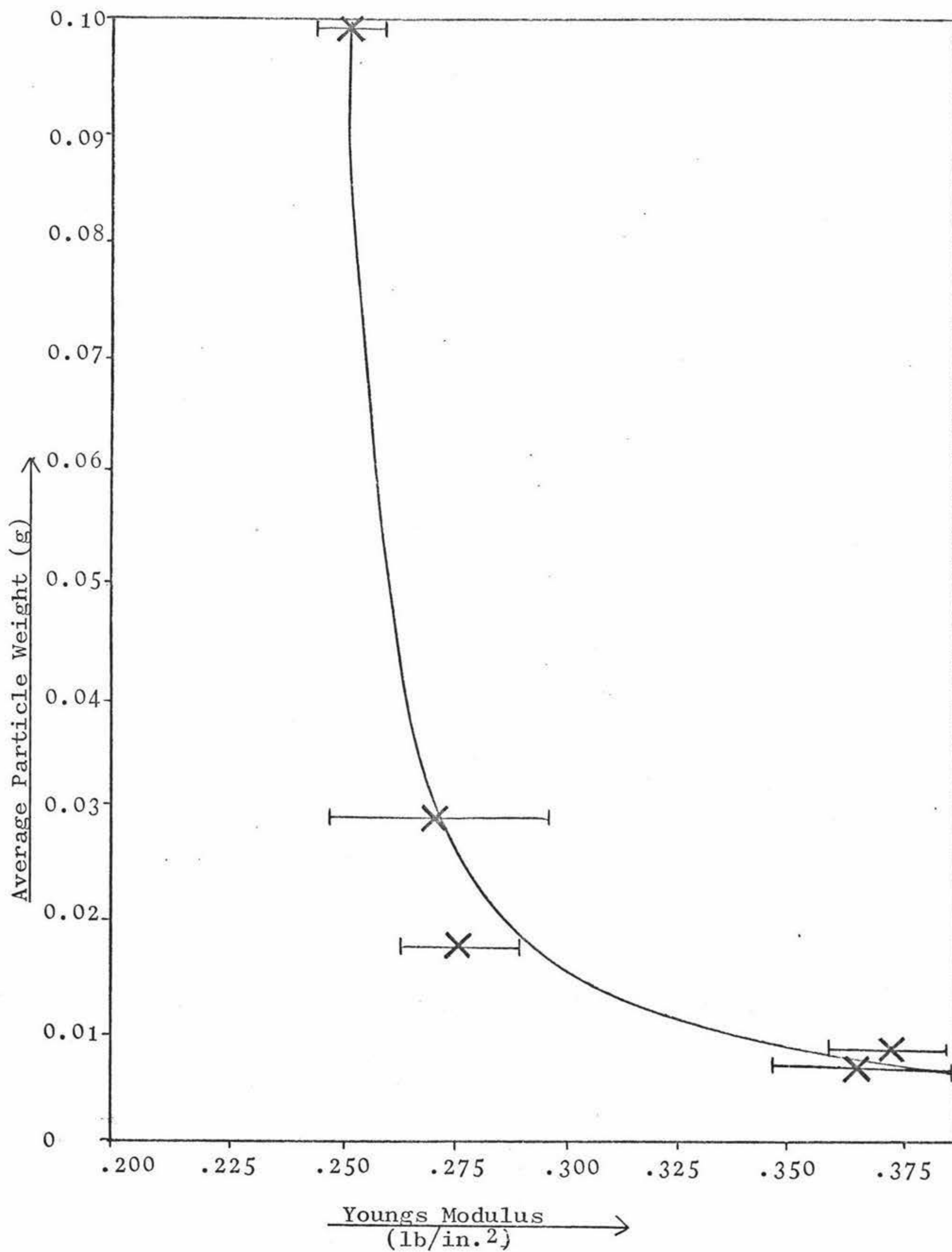


FIGURE 3:23 The effect of particle size on the Average Break Force required to rupture samples of restructured fish mince

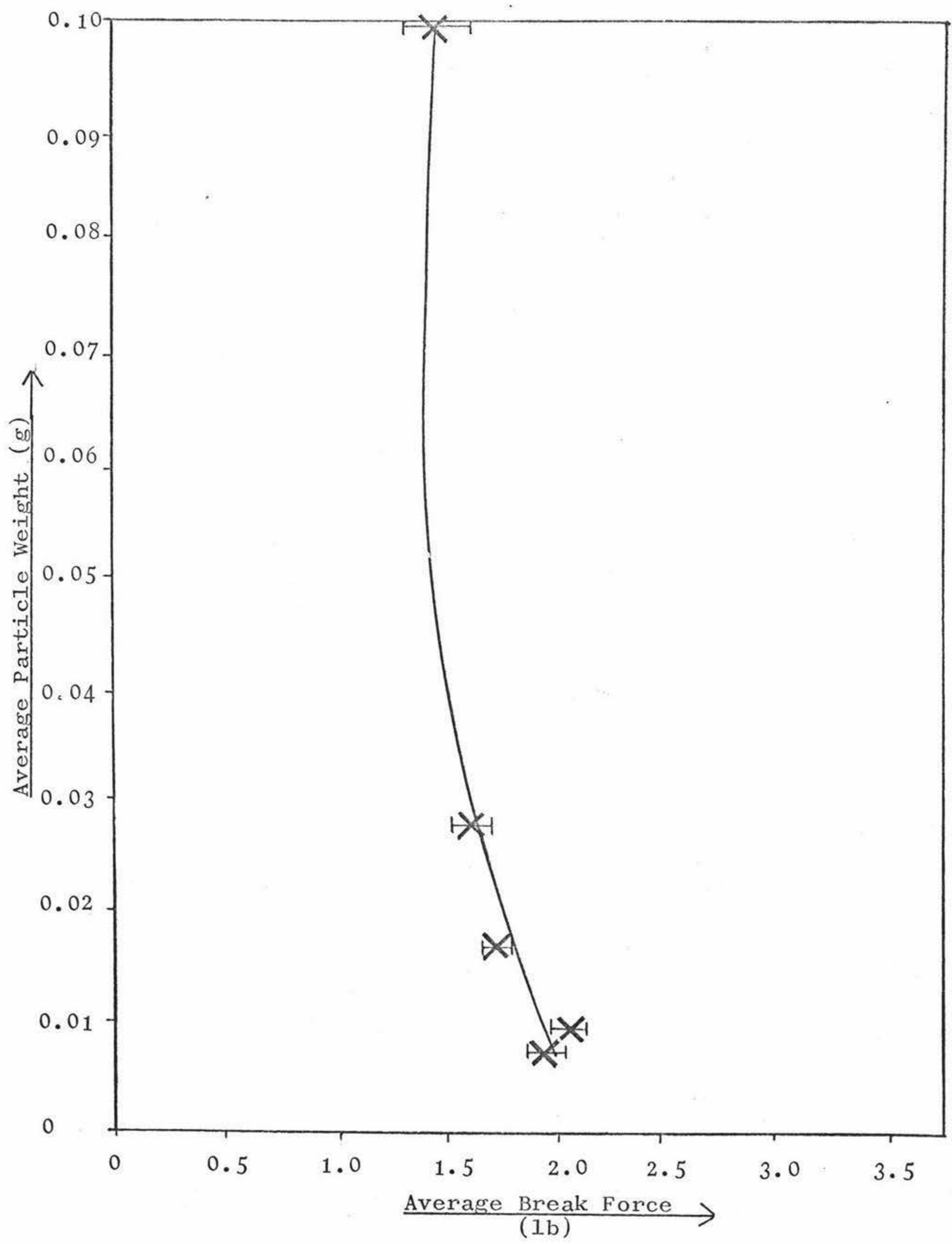
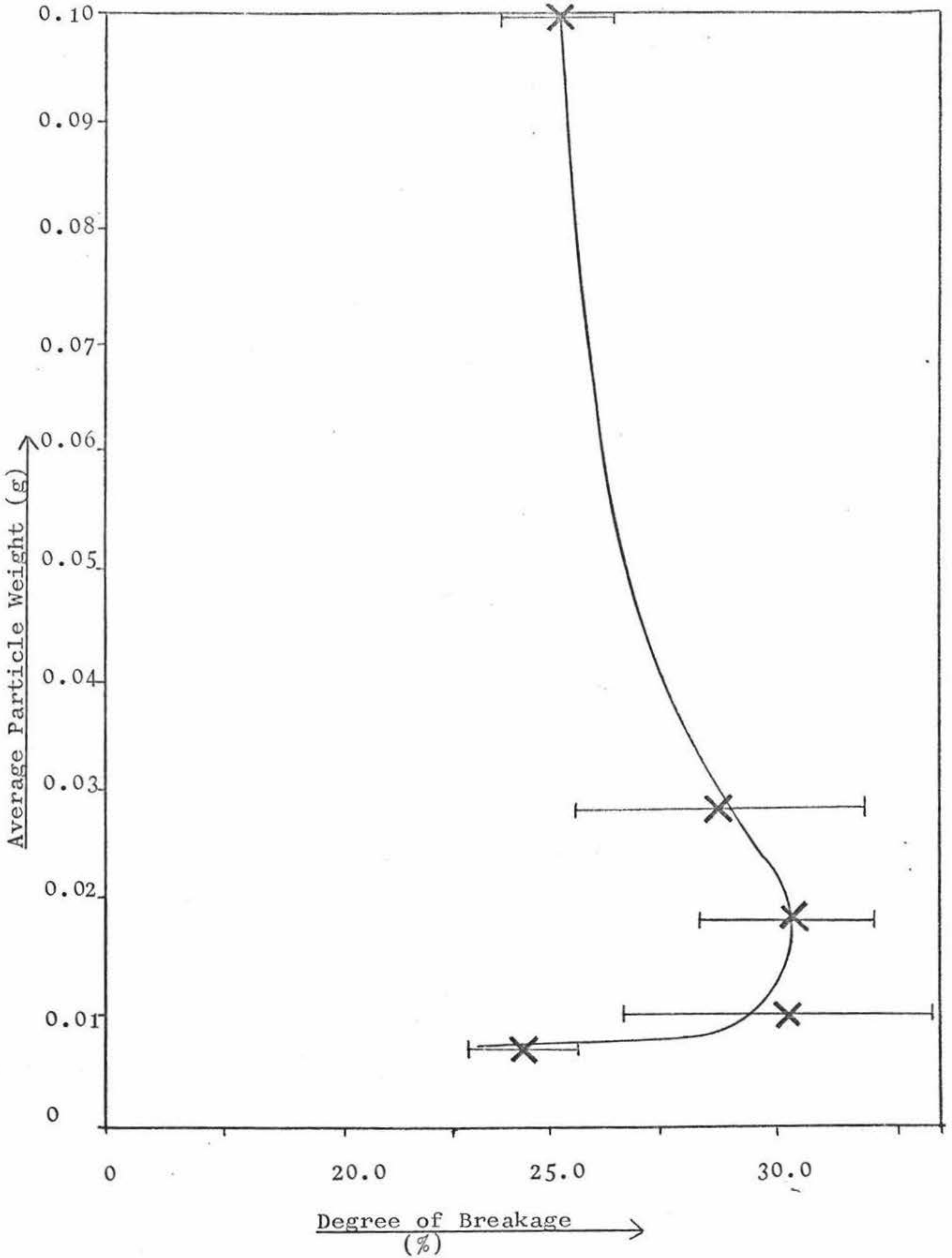


FIGURE 3:24 Effect of particle size on the crumbliness of the restructured fish mince



The crumbliness of the product, as measured by the Degree of Breakage, is seen to increase with the decrease in particle size, to a maximum of 0.015 g. It is suggested that the crumbliness increases with the greater number of particles, until at a given point the effect of the protein extracted, which increases as the product is ground down, is such that the gel characteristic becomes dominant whereas before each of the large number of small particles represented a zone of weakness in the gel structure. It can be seen that as the product is ground down from an average size of 0.015 g, so the Degree of Breakage again drops off very rapidly.

Conclusions

Youngs Modulus, the Average Break Force and the Degree of Breakage, as derived from the OTMS compression curves of the heat gelled fish mince showed that the texture of the product in terms of resilience or chewiness, binding strength and crumbliness, depends upon the combined effects of these characteristics. By varying certain conditions, in particular by the addition of salt, the characteristics of the restructured product can be modified. In these modifications it was found that the resilience, binding strength and crumbliness do not necessarily follow the same pattern.

The addition of salt up to 0.4% to trevally mince resulted in no change in the crumbliness, a drop in the resilience of the product, with a simultaneous but smaller drop in the binding strength. These changes are thought to be brought about by the formation of a heat set gel from the soluble myofibrillar proteins, this gel being increasingly

diluted with moisture extracted from the milieu by osmotic forces resulting from the addition of the salt.

When salt is added to the mince in excess of 0.4% the restructured product showed increasingly strong binding characteristics. This can be seen in the high Average Break Force required to rupture the sample (in excess of 3.5 lb), and the low Degree of Breakage or crumbliness (less than 15%). These characteristics were undesirable in that they do not resemble those of cooked fish fillet, which has a high Degree of Breakage, and ruptures under very low forces. In this case it is postulated that salt concentrations in the system have increased to the point where significant solubilisation of the myofibrillar protein is occuring, thus giving rise to a more highly concentrated protein solution which sets to gels having the characteristics observed.

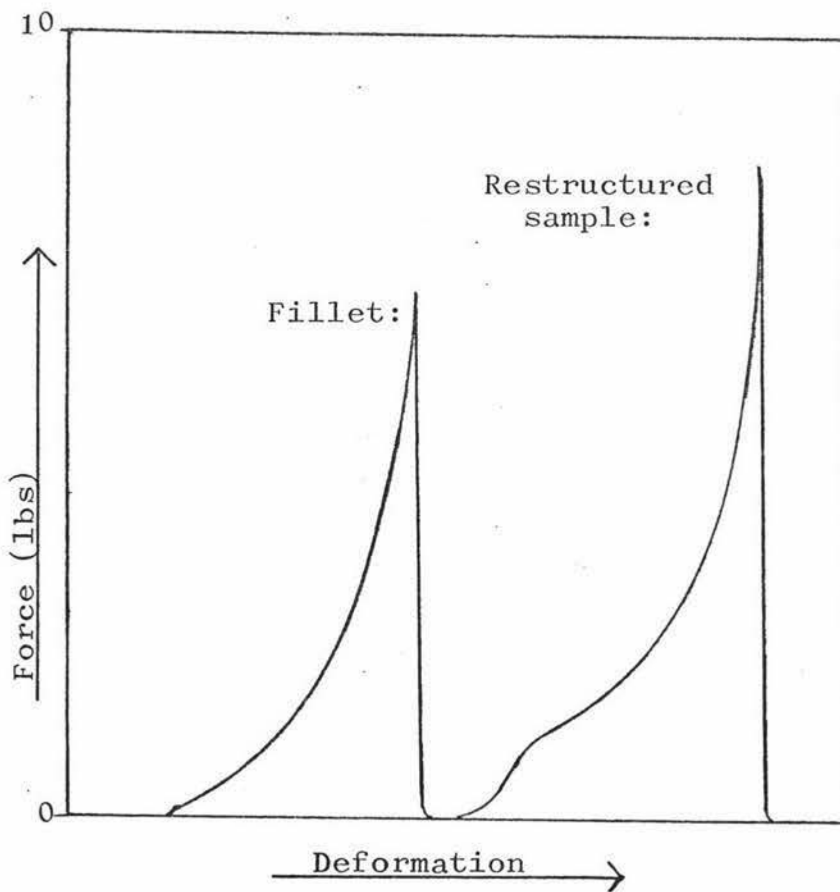
The addition of water was found to result in a weaker structure being formed and was desirable as the restructured product had a dry mouth feel when no water was added.

Phosphates appear to be effective only when used on fresh or good quality fish. The reason for this is not known, but it is thought that poor quality fish in which phosphates have no effect were in a condition in which the protein was already partially denatured.

The sample which was found to have textural characteristics most closely resembling that of cooked fillet was prepared from gurnard mince. The mince had been recovered

from a meat/bone separator and was beaten in the presence of 1% of salt and 12% added water. The OTMS curves for this product and for the cooked trevally fillet are compared in Figure 3:25.

FIGURE 3:25 Comparison of OTMS compression curves of samples of cooked trevally fillet and restructured gurnard mince containing 1% added salt and 12% added water



SECTION IV

Development of oxidative rancidity in frozen trevally products

In earlier work (Sorensen 1971b) it was found that fish finger slabs prepared from trevally developed an unpleasant odour and taste after as little as four weeks storage at $-18^{o}C$ ($0^{o}F$). This change was not noticed in slabs prepared from gurnard, and did not occur if the trevally slabs were packed in heat-sealed polythene bags. Although no chemical tests were carried out at that time it was thought that the unpleasant flavour which occurred in the trevally slabs was due to autoxidative rancidity.

The lipids in fish muscle differ from those of most other animals in their high content of polyunsaturated fatty acids. In fat fish such as trevally, this highly polyunsaturated fat content results in the fish turning rancid even after a short period of storage (Bosund and Ganrot 1969). In addition to the unsaturated nature of their fish oils, dark fleshed fish are known to contain relatively high contents of hematin compounds. These compounds, which are largely made up of hemaglobin, myoglobin and the cytochromes are strongly pro-oxidant. Storage of frozen fish, poultry and meats is often limited by hematin catalysed rancidity (Tappel 1962).

Hematin catalysed auto-oxidation can be very difficult to control. Chelating compounds such as citric acid and EDTA which are known to have an inhibiting effect on metal catalysed oxidative reactions have no effect over oxidation reactions catalysed by the hematin compounds (Tappel 1962).

Mild heating is often used to inactivate lipase enzymes. A similar treatment has been reported to increase the activity of hematin catalysed oxidation (Watts 1962).

Antioxidants such as polyphenolic antioxidants which can retard the initial formation of lipid peroxides are not permitted for use in fish products. Even if legislation were modified, the incorporation of small amounts of oil soluble antioxidants into the predominantly water soluble fish product is a difficult exercise in practice, and for this reason is not always effective.

The autoxidation of an organic compound normally involves the addition of a molecule of oxygen to a C-H bond of a hydrocarbon chain, to give a hydroperoxide of the general formula ROOH. Thus an essential requirement for the autoxidation reaction to occur is the presence of oxygen. In practice, hematin catalysed autoxidation is most effectively inhibited by excluding oxygen from the surface of the fish. This is normally achieved by glazing, using water often incorporating a stabilizer such as sodium alginate. The glaze may contain an added antioxidant such as ascorbic acid for increased effectiveness (Anderson and Danielson 1961).

Preparation of Samples

The six samples summarized in Table 4:1 were prepared from trevally fillets. The fish used in this experiment had been freshly processed in Auckland, where it was frozen and sent directly to Massey University.

The fish was tested upon arrival and the peroxide value was found to be in the range 3.5 - 3.6 milliequivalents

oxygen per kilogram of fat. An organoleptic assessment of the quality of the fish was also carried out on samples which had been steamed for twenty minutes. Although the fish did not taste particularly fresh, no unpleasant odour or flavours typical of autoxidation were detected.

TABLE 4:1 Summary of the products stored at -18°C (0°F) for autoxidative studies

Sample No.	Product	Storage conditions
1	Fresh trevally fillet	Unsealed polythene bag
2	Fresh trevally mince	Unsealed disposable petri dish
3	Fresh trevally mince	Sealed disposable petri dish
4	Cooked mince	Unsealed disposable petri dish
5	Cooked mince	Sealed disposable petri dish
6	Cooked mince with 0.05% ascorbic acid	Sealed disposable petri dish

Fillet samples were individually frozen, and stored in unsealed polythene bags for the duration of the experiment.

Minced samples were prepared from skinned and boned fillet ground in a food mincer fitted with a 5 mm plate. Fresh mince was filled directly into 90 mm disposable petri dishes, and cooked samples were prepared by steaming the product for twenty minutes to give a heat treatment similar to that shown in Figure 3:1.

Unsealed samples refer to those products in petri dishes with lids fitted, but with no seal between the lid and

the base, whereas the sealed samples were sealed with an adhesive tape.

Determination of Hydroperoxides

Hydroperoxides are the first measurable compounds to be formed during the oxidation of fat, and although they are themselves considered not responsible for the unpleasant flavours found in rancid products, hydroperoxide levels are widely used as an index of the extent of oxidation in food products (Pearson 1970).

In this experiment the hydroperoxides were determined by a modified form of the Loftus Hills and Thiel ferric thiocyanate peroxide test reported by Hollaway (1966). The modifications developed by Wilson (1973) were finalised in this work and enabled samples of fish to be tested directly. An added advantage of the modification was that peroxide values could be found from as little as 0.01 g extracted oil.

Reagents:

All chemicals were Analytical Reagent Grade:

Chloroform

Methanol

Barium chloride $BaCl_2$, $2H_2O$

Ferrous sulphate, $FeSO_4$, $7H_2O$

Hydrochloric acid, 10N solution

Ammonium thiocyanate

Sodium chloride

Anhydrous Sodium sulphate

Bright iron wire

Hydrogen peroxide 100 vol.

Solutions:

1. Solvents: Chloroform - methanol 2:1 (V/V)
Chloroform - methanol 70:30 (V/V)

2. Ferrous chloride solution: Barium chloride (0.4 g) is dissolved in 50 ml glass distilled water, 2.0 ml of 10N hydrochloric acid is then added. Barium sulphate is precipitated out and is removed by centrifuging. The clear solution is decanted into a bottle protected from light. The solution remains stable for only one week.

3. Ammonium thiocyanate solution: 30 g ammonium thiocyanate is dissolved in distilled water and the volume made up to 100 ml.

4. Sodium chloride solution: 9 g sodium chloride is dissolved in distilled water and the volume made up to 100 ml.

5. Standard ferric iron solution: 0.500 g of bright iron wire is dissolved in 50 ml of 10N hydrochloric acid and oxidized with 1 to 2 ml hydrogen peroxide. Excess peroxide is removed by boiling and the solution is diluted to 500 ml.

Procedure

1. Extraction of oil: appromately 20 g of the fish sample is placed in an atomizer jar. 100 ml 2:1 V/V chloroform-methanol is added, and the sample is agitated for 2 to 3 minutes or until the fish is disintegrated to a smooth slurry. The filtrate is recovered using a Watman No. 1 filter paper and a Buchner funnel.

2. Washing out protein residues: Trace amounts of protein in the filtrate cause cloudiness and interefere with subsequent

readings. These proteins are removed by washing the filtrate twice with 30 ml 0.9% sodium chloride solution in a 100 ml separating flask. After the first wash, the top aqueous layer is drawn off with a Pasteur pipette fitted to a vacuum line. The second wash is then performed and after the layers have separated the bottom layer containing the oil in chloroform is run into a 150 ml Erlenmeyer flask.

3. Removing traces of moisture: Trace amounts of moisture remaining after the washing process are removed chemically by the addition of approximately 5 g anhydrous sodium sulphate. The removal of moisture is complete when the oil in chloroform solution is clear.

4. Drying off the chloroform and weighing the oil: The supernatant fluid is qualitatively transferred from the Erlenmeyer flask to a 100 ml Quickfit flask which is fitted to a Rotary evaporator, where the volume of chloroform is reduced to about 3 ml under vacuum.

The solution remaining in the flask is poured into a clean, dry, tared weighing bottle, and the remainder of the chloroform is evaporated off under a stream of nitrogen gas, with the container resting in a water bath set at 70°C, the flow of nitrogen gas being maintained until the sample is dry when the top of the container is carefully replaced.

The outside of the bottle is dried and the bottle reweighed to find the weight of the oil.

10 ml 70:30 V/V chloroform-methanol is added to the weighing bottle and the fat is redissolved. It is important that care be taken to ensure that all the oil is redissolved,

as small quantities of undissolved oil can give rise to large errors.

5. Preparation of samples to read optical density: The quantity of sample required will depend on the weight of oil and the peroxide value of the sample. It is convenient to pipette 0.5, 1.0 and 2.0 ml of the redissolved sample into 19 x 150 mm test tubes and to make up to 9.9 ml with 70:30 V/V chloroform-methanol solution.

Make up a reagent blank containing all the reagents except the fat extract and a fat blank containing all the reagents except the ferrous chloride.

To the 9.9 ml of fat and solvent add 1 drop of ammonium thiocyanate, followed by 1 drop of ferrous chloride solution and mix.

The test samples and the two blanks are held for 5 minutes in subdued light and the colour intensities are read at 505 μm in a suitable colorimeter or spectrophotometer.

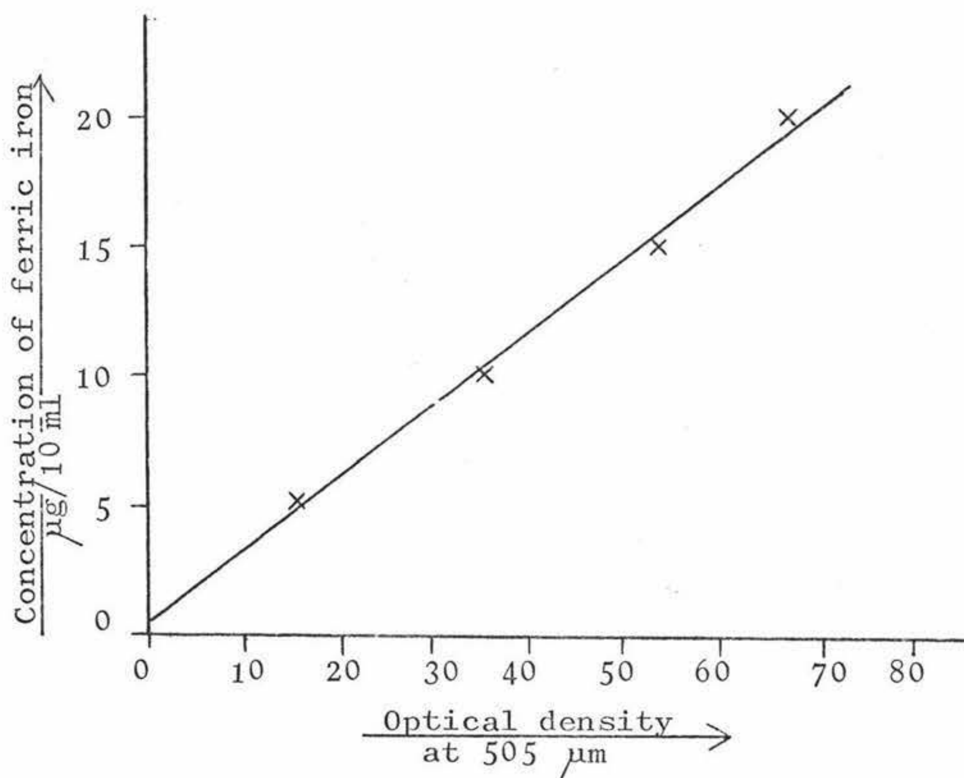
Further dilutions of the fat may be necessary depending on the peroxide value of the initial test run.

Optical density reference curve

Dilute 1.0 ml of the 1.0 mg/ml aqueous iron solution to 100 ml with 70:30 V/V chloroform-methanol. With this solution together with appropriate amounts of the chloroform methanol solvent, prepare four tubes containing 5, 10, 15 and 20 μg of ferric iron in 9.9 ml of the chloroform methanol solvent. Add 1 drop of ammonium thiocyanate solution and 1 drop of water containing 2 ml of 10N hydrochloric acid per 100 ml.

Mix, allow the colours to develop for 5 minutes and measure the absorbance (optical density) of the reference solution using the same procedure as for the test sample. Plot the reference curve, optical density vs concentration of ferric iron as shown in Figure 4:1.

<u>FIGURE 4:1</u> <u>Standard reference curve showing typical relationship between optical density and concentration of ferric iron</u>



<u>Note</u>: The reference curve used in the experiment was a regression line drawn after the four reference points had been measured at three different times.

<u>Calculation of Peroxide Value</u>

The peroxide value is expressed as millequivalents of oxygen per kilogram fat. The concentration of the unknown and blanks are calculated in terms of µg of iron/10 ml and the nett figure for the unknown is then obtained by deducting the

blanks.

$$PV = \frac{\text{Nett } \mu g \text{ iron/10 ml}}{\text{Weight of fat in grams x 55.85}}$$

Results and Discussion

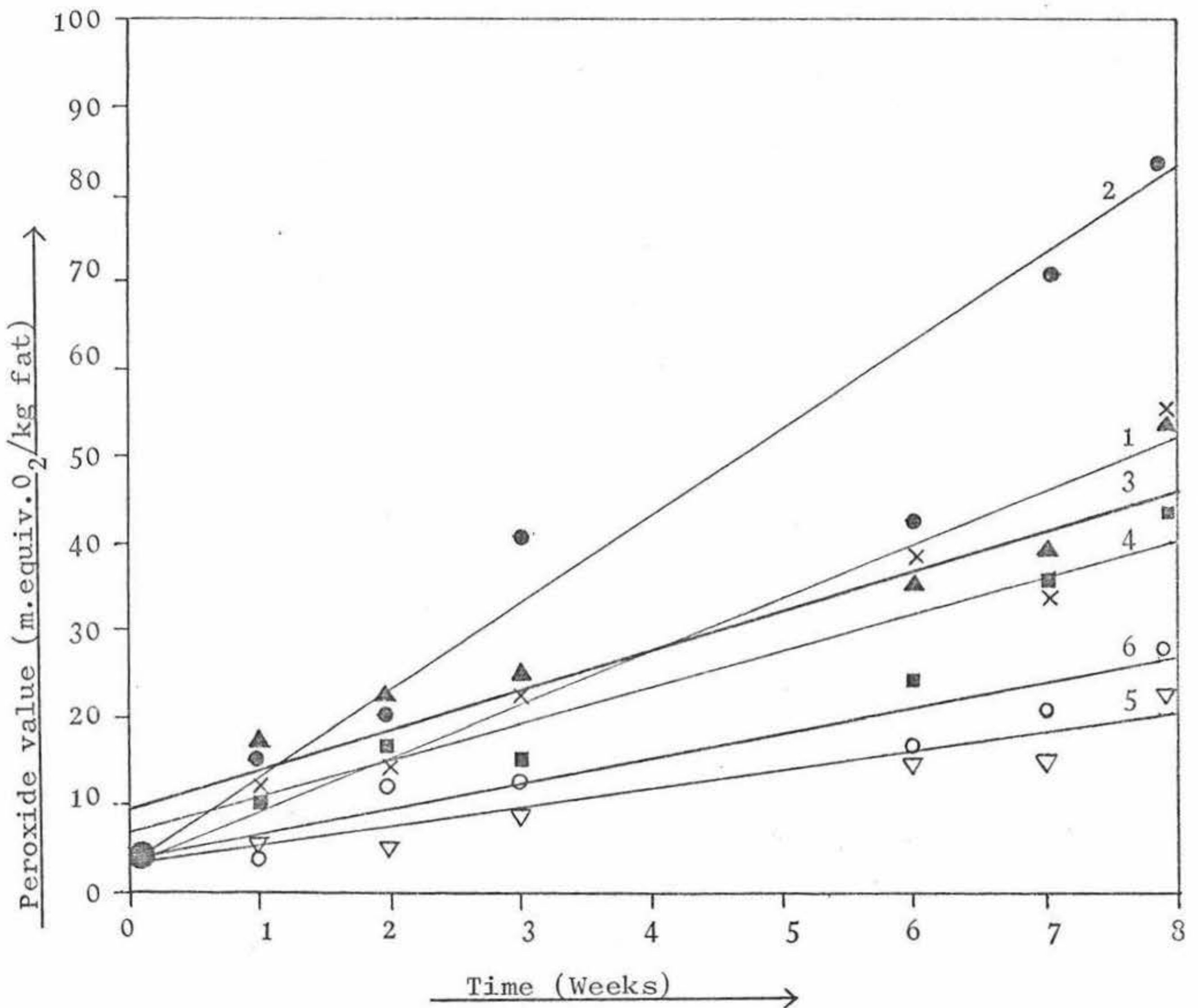
The frozen samples were tested over an eight week period, and the peroxide values found during this time are plotted in Figure 4:2. As the results show no significant lag phase or levelling out during the experiment, the data was treated as though a straight line relationship existed between peroxide value and time. The lines drawn in Figure 4:2 are regression curves of best fit.

The rate of oxidation can be found from the slopes of the curves shown in Figure 4:2, and have been summarised in Table 4:2.

TABLE 4:2 Rates of Oxidation of the frozen samples Stored at -18°C (0°F)

Sample	Packaging	Rate of Oxidation m.equiv.O_2/kg fat/week
Trevally fillet	Frozen individually in plastic bag	8.86
Raw mince	Unsealed petri dishes	5.20
Cooked mince	Unsealed petri dishes	4.63
Raw mince	Sealed petri dishes	3.42
Cooked mince	Sealed petri dishes	4.11
Cooked mince + ascorbic acid	Sealed petri dishes	2.76

FIGURE 4:2 Relation between oxidation (peroxide value) of the frozen fish samples and time



KEY:

1. × Fresh unsealed mince
2. ● Fresh fillet
3. ▲ Cooked unsealed mince
4. ■ Cooked sealed mince
5. ▽ Cooked sealed mince with 0.05% added ascorbic acid
6. ○ Sealed fresh mince

Statistical Analysis of Results

Each group of results was analysed statistically in order to find the significance between treatments. This was done by an analysis of variance to find the 95% confidence limits about each regression line. The curves obtained for two of the results, the fish fillet and the cooked sealed mince with 0.05% ascorbic acid added are shown in Figure 4:3.

Effect of Treatments

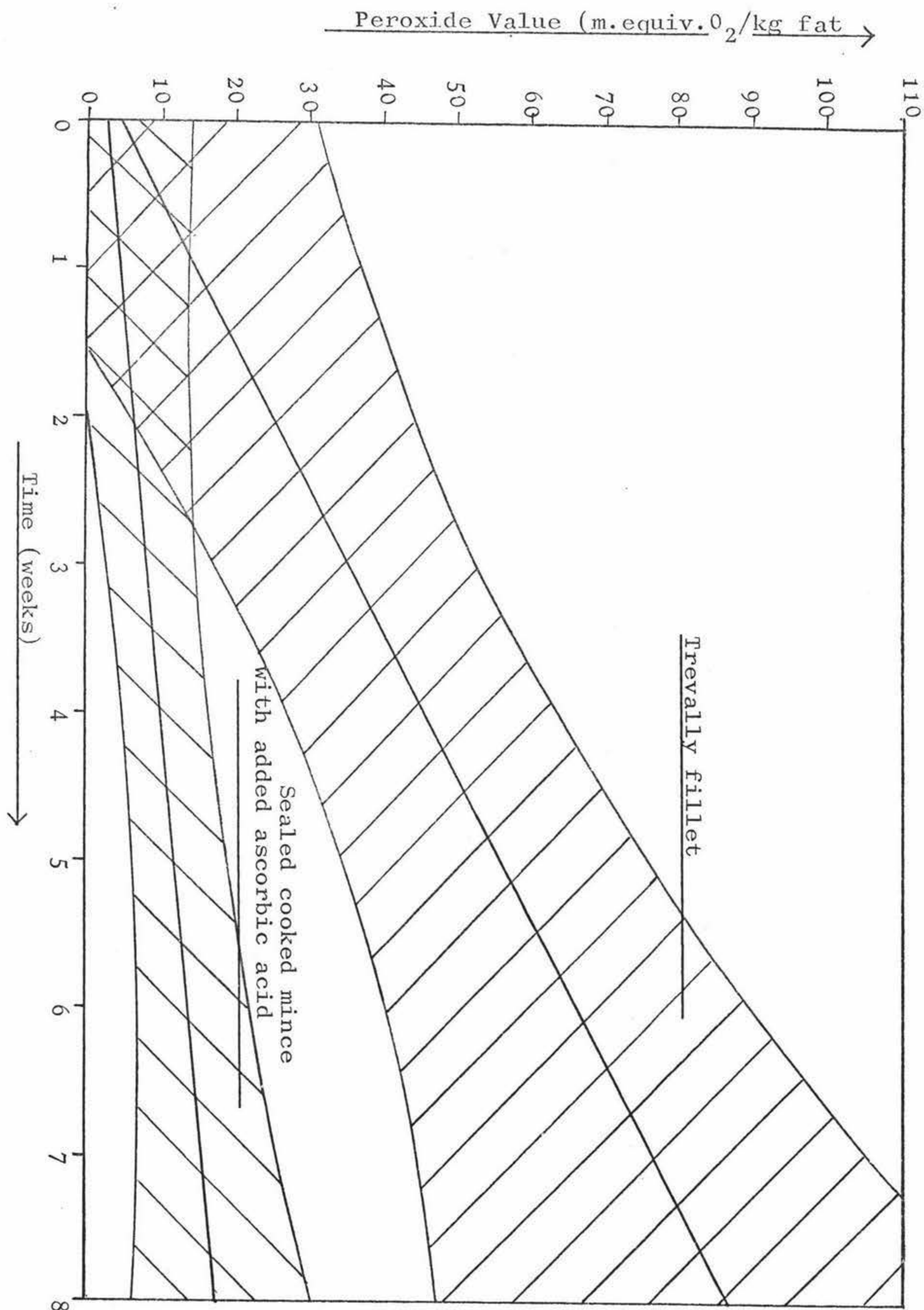
(a) Condition of storage

The samples were stored in three different conditions as identified in Table 4:2 from which it can be seen that the rate of oxidation is appreciably higher in the individually frozen fillet than in any of the mince samples which were packed in either sealed or unsealed petri dishes.

Statistical analysis of the results shows that the differences between the frozen fillets and the mince in unsealed containers are not significant at a 95% confidence level. This is due to the scatter of the results and because only seven samples of each product were tested during the storage trial. The apparent differences in the rates of oxidation between these samples may have become significant had the storage trial been carried over a longer period of time, but obvious deterioration of samples was already becoming apparent and further testing would thus not have been relevant.

A comparison between the fillet and the sealed samples on the other hand shows that the peroxide values of the fillet are significantly higher (95% confidence limits) than the peroxide values of both the sealed fish mince and the sealed

FIGURE 4:3 Comparison between the regression lines with 95% confidence limits for trevally fillet and sealed cooked mince with added ascorbic acid



cooked mince with 0.05% added ascorbic acid after three weeks. The rate of oxidation in the sealed cooked mince appeared slightly higher than in the other sealed samples, and the peroxide values of the fillet are not significantly higher than the values in the sample until after six weeks storage.

The peroxide values of the unsealed cooked and unsealed raw mince samples were also significantly (95% confidence) higher than the values of the sealed raw mince and sealed cooked mince with the added ascorbic acid after six weeks storage. There was no significant difference between the unsealed samples and the sealed cooked mince.

These results suggest that the rate of oxidation of the oil in fish products increases with the availability of air or oxygen. This conclusion has also been reached by workers working on products other than fish (Lundberg 1962), and is consistent with the current understanding of autoxidative reactions.

It is therefore concluded that the exclusion of air or oxygen is essential if the product is to have an acceptable storage life.

The restructured fish material has been developed for use in battered and crumbed products such as fish fingers. In this operation the batter forms a continuous coating about the product, and can have a protective effect similar to glazes which are often used on frozen fish. For the batter to be effective in this regard it is important that the coating is impermeable to air under freezer conditions and that the batter does not have a tendency to crack. To ensure these requirements

are satisfied, work may have to be done on formulations incorporating stabilizers such as sodium alginate and sodium carboxymethylcellulose.

The addition of 0.05% ascorbic acid to glazes used on fish fillets have been reported to be very effective in prolonging the storage life of the frozen product (Olcott 1962). A similar effect may be found by adding the ascorbic acid to the batter.

Although the rate of autoxidation was found to be significantly lower in the sealed samples, there is still a positive increase in the peroxide values of these products with time. By tasting portions of the sample used for peroxide evaluation, it was found that the product became unacceptable when the peroxide value exceeded a value of 30 milliequivalents of oxygen per kilogram oil. At this level the product did not taste rancid, but a slightly rancid odour was apparent. This value is confirmed by Pearson (1970) who reported that rancidity is not usually detected before the peroxide value reaches 20-40 milliequivalents of oxygen per kilogram of oil.

Analysis of the results (Figure 4:2) shows that even the sealed cooked mince with 0.05% added ascorbic acid which was the most stable product, had a storage life of only 11 weeks (8 - 14 week range). The oxidation of the sealed product is probably due to air incorporated in the mince during its preparation and mixing in of ingredients. Redox values of the mince were found to vary from +4.8 mv before mixing to +6.2 mv after mixing. This shows the need to mix the product in the absence of air. Where conventional mixers are used it may be

possible to shield the product from air under a cloud of carbon dioxide or nitrogen gas. Alternatively, dry ice (carbon dioxide) could be evaporated in the mix. Used in this form carbon dioxide would have the added advantage of keeping down the temperature of the products.

(b) Effect of cooking

Mild heating of products such as meat and fish has been reported to promote oxidation while more intensive heating can have an inhibiting effect on the rate of oxidation (Watts 1962).

The rate of oxidation in the sealed samples was slightly higher for the cooked sample than for the raw sample, while in the unsealed samples the converse was found. However, neither of these sets of results showed a statistically significant difference between the cooked and raw samples. Thus it cannot be concluded that heating the samples to an internal temperature of 80°C had any significant effect upon the rate of autoxidation during storage.

(c) Effect of the addition of 0.05% ascorbic acid

The use of ascorbic acid to limit autoxidative deterioration is widely reported in published literature (Olcott 1962), where it is described as having varying degrees of effectiveness when used on fish products.

In this experiment the difference between the cooked sealed sample and the cooked sealed sample with the added ascorbic acid was not significant over the duration during which samples were taken. However the difference in trend and the narrow margin by which the confidence limits of these

two curves overlap suggest a significant difference may have become apparent if the experiment had been continued for a longer period of time.

Further work is therefore necessary before the beneficial effect of ascorbic acid can be ascertained with complete certainty but the data suggests it could probably be incorporated, with beneficial effects upon the storage life of the restructured products.

Any beneficial effect of the ascorbic acid used in this way is thought to be due to its ability to chemically complex the oxygen which has become incorporated in the product during its production.

Because of its high oil content and the presence of the haematin-type oxidation catalyst it may be difficult to achieve satisfactory storage at -18°C of products prepared from trevally, even if care is taken to exclude oxygen by the means described above. The higher the storage temperature, the greater the acceleration not only of the chain propagation reactions, but also the peroxide decomposition and thereby a greater concentration of free radicals becomes available for the initiation and propagation of new reaction chains (Lundberg 1962). However, since the converse is also true if the temperature of storage can be reduced, then better storage results may be achieved in the -29°C (-20°F) to -40°C (-40°F) range. This will need investigation to establish if it has a significant effect upon the storage life of the product. Its practical implementation within the factory and in the distribution system will need to be examined for feasability if improved storage is shown to result at these low temperatures.

SECTION V

Microbiological examination of raw materials used in the preparation of the restructured fish, and an evaluation of the microbiological effectiveness of the heat used in the process

The aim of the work reported in this section was to examine the microbial quality of the raw materials used in the preparation of the restructured product. The effect of storing these materials over a twenty-four hour period at $0^{o}C$ and at $10^{o}C$ was also investigated. Similar examinations were carried out on the cooked slabs of gelled mince to find the effectiveness of the heat process in reducing the microbial level in the restructured product.

Materials Sampled

The fish used in this experiment were headed and gutted trevally, which had been processed in February 1973, during the peak of the fishing season. The sample was taken from a stock of frozen fish which had been set aside to be processed into fish fingers during the winter months.

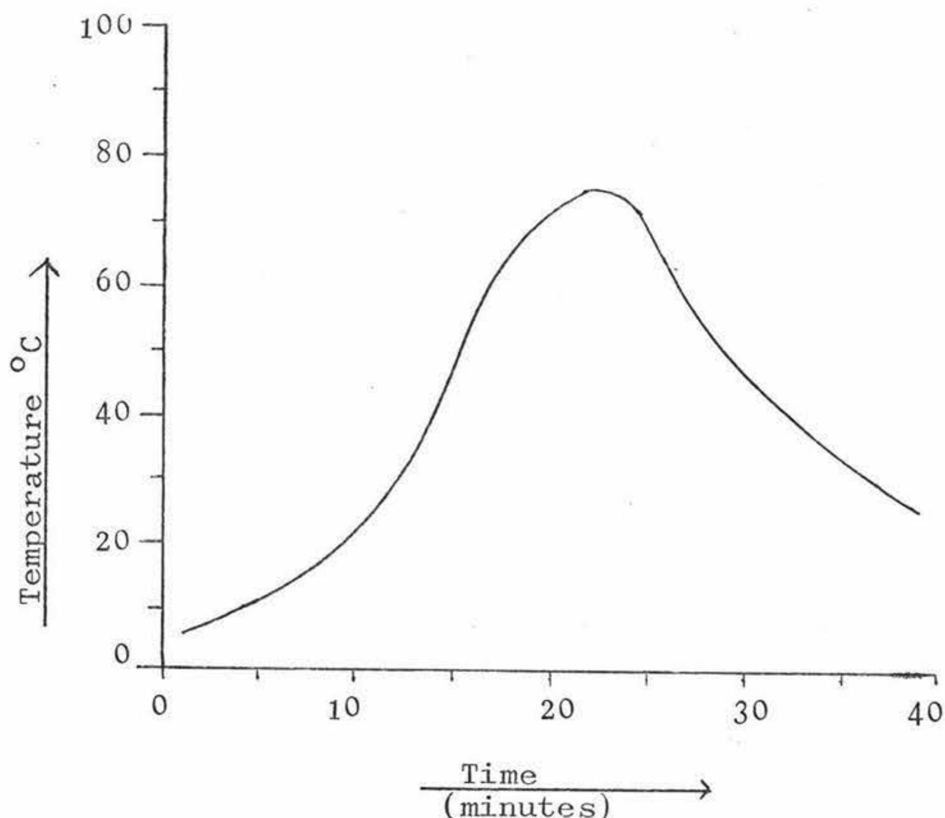
Fillets were prepared in the usual way, the fillet being removed from behind the head and the vent, and out from the backbone.

As a meat/bone separator was not available for this work, the mince was prepared from skinned and boned fish, which was passed through a mincer fitted with a 5 mm plate.

The cooked samples were prepared from fish mince, previously treated with 0.75% sodium chloride, by steaming for twenty minutes. The internal temperature during cooking was

measured using a Varian millivolt recorder with the thermocouple placed in the centre of the sample. The temperature profile for the cooking process is given in Table 5:1.

FIGURE 5:1 Temperature profile, showing the temperature at the centre of the product during 20 min. steaming and subsequent cooling:



Method of Microbial Assessment

The microbiological evaluations were determined by pour plate techniques. Culture media were used as outlined below. Media were prepared from standard dehydrated culture media according to manufacturers instructions, and a 0.1% solution of peptone water was used for the dilutions outlined in Figure 5:1.

After the media were made up, they were sterilized in the autoclave for 15 minutes at 15 pounds pressure (120°C). An exception to this was violet red bile agar which was brought to boiling point, cooled to 42°C and used directly.

Culture media used:

Nutrient agar

Total counts were evaluated on nutrient agar. Two duplicate sets of plates were prepared, and were incubated at 25°C and 10°C. The 25°C plates were incubated for three days and the 10°C plates for five days before counting the colonies.

Violet red bile agar

Violet red bile agar was used for direct plate counts of coliforms. The coliforms were identified as those colonies 1-2 mm in diameter, having a purplish red colour and surrounded by a reddish zone of precipitated bile. Colonies were counted after incubating the plates at 37°C for 18-24 hours.

Mannitol salt agar

Mannitol salt agar is a selective medium for the isolation of pathogenic staphylococci, and was used for direct plate counting of these organisms. Colonies were counted after incubating the plate at 37°C cor 24-36 hours. Both those colonies surrounded by a yellowish zone, and those colonies about which the immediate zone was indistinct, were presumed to to pathogenic staphylococci.

Procedure

Raw material quality

The bacteriological quality of the raw material was evaluated after thawing the frozen fish overnight (12 hours). The samples were filleted and minced as indicated in the "Materials Sampled".

In addition to the bacteriological examination, an organoleptic quality assessment was carried out on samples of fillet steamed for twenty minutes.

Evaluation of growth rate

The materials were sampled at intervals of $2\frac{1}{2}$ to 3 hours for the first 12 hours, and again after 24 hours. Growth curves were determined for fish and fish mince stored at $0^{o}C$ and at $10^{o}C$.

Preparation of Sample

Samples were prepared by accurately weighing approximately 20 g of the specimen into a sterile atomizer jar. The amount of peptone water required to make a 10:1 dilution of the sample was then measured in, and the sample was agitated for 2-3 minutes.

Dilutions for plating were prepared as outlined in Figure 5:2. Plates were prepared in duplicate for all tests.

Results, which are presented in the following section, were the mean value of two plate counts, using only those dilutions giving counts between 30-300 per plate.

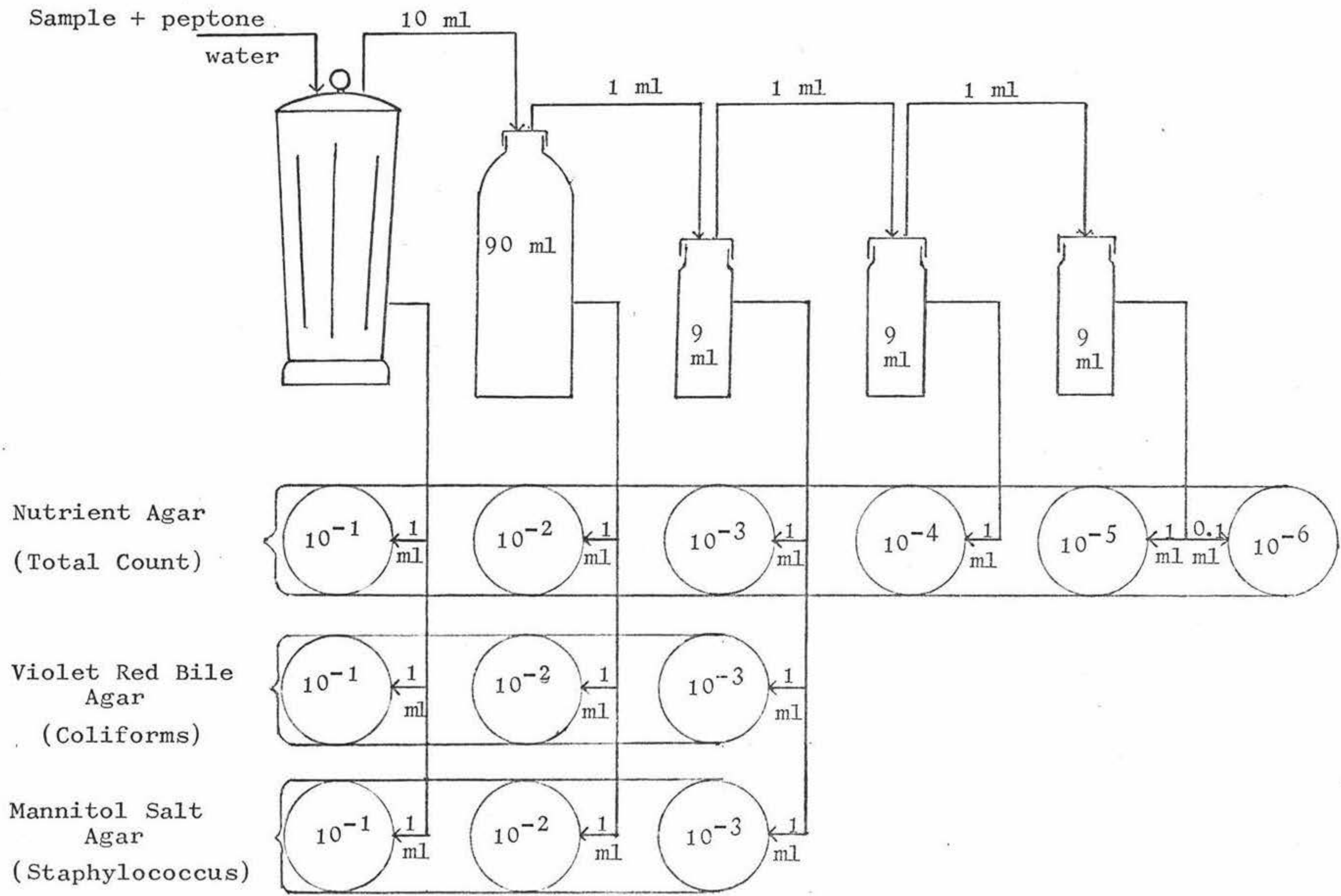


FIGURE 5:2 Scheme used in evaluating the bacteriological quality of the fish sampled

Results

Raw material quality

Two samples of the raw materials were evaluated. The result of the bacteriological examinations shown in Table 5:1 is the average value of the two samples tested.

TABLE 5:1 Bacteriological quality of the raw fish materials

	Number of organisms per gram	
	Trevally fillet	Trevally mince
Total count (25°C)	3.7×10^5	1.15×10^5
Total count (10°C)	1.0×10^5	2.8×10^4
Staphylococci	4.2×10^3	4.0×10^3
Coliforms	8.0×10^3	3.9×10^3

Growth curves

The results of the growth curves of the raw fish material are plotted in Figures 5:3 - 5:6. Growth rates of bacteria were also examined in the cooked product stored at 0°C and at 10°C. It was found that there was no significant change in bacterial number over the twenty four hour test period, and no growth curves have been prepared.

Effect of heat

The heat applied during cooked (Figure 5:1) was effective in reducing the number of viable organisms in the product. The bacterial numbers shown in Table 5:2 are the average value for eight separate samples of the cooked product.

FIGURE 5:3 Bacterial growth curves from raw fish materials: Total count, Incubated 25°C

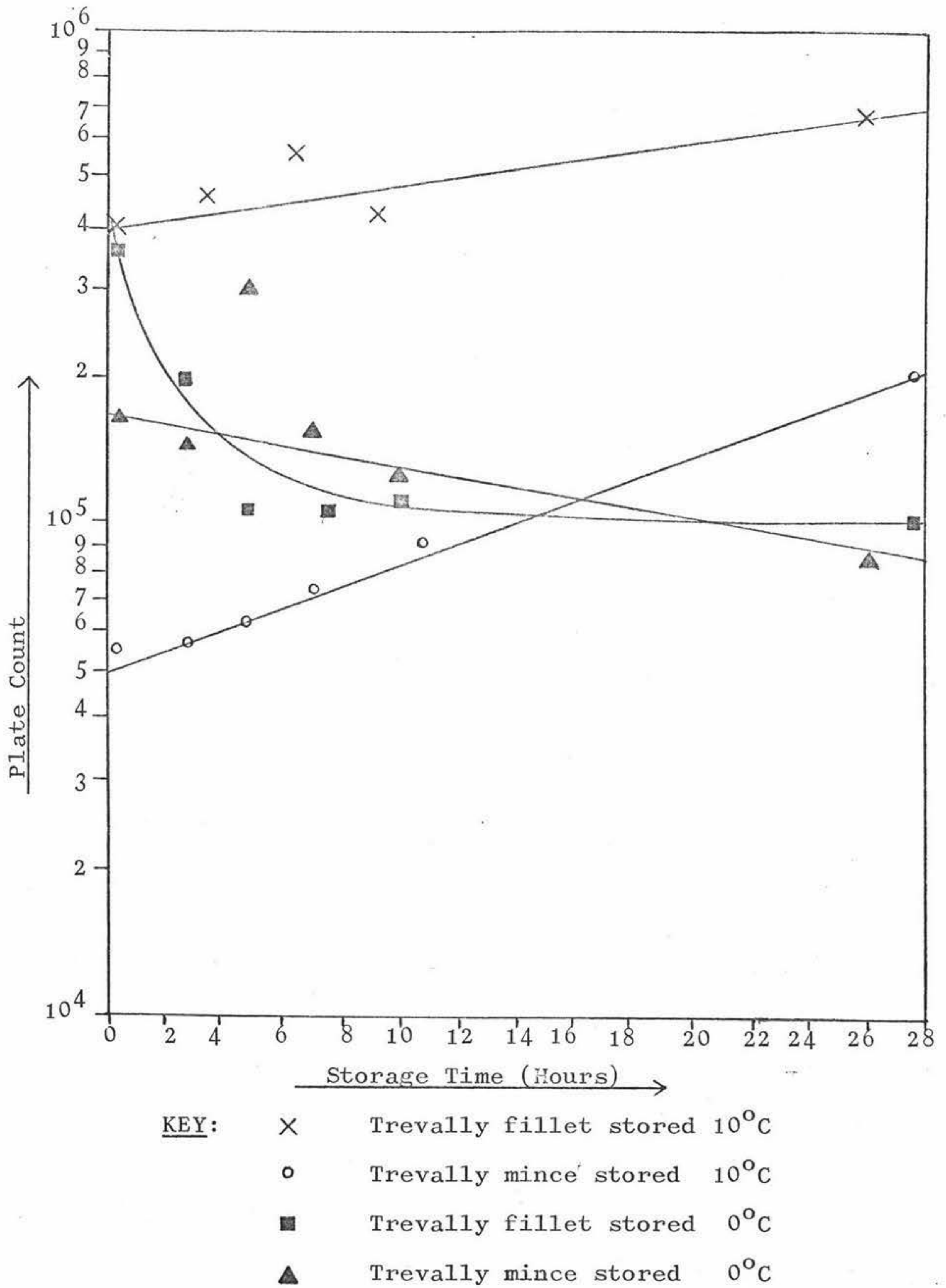


FIGURE 5:4 Bacterial growth curves from raw fish materials: Total Count, Incubated 10°C

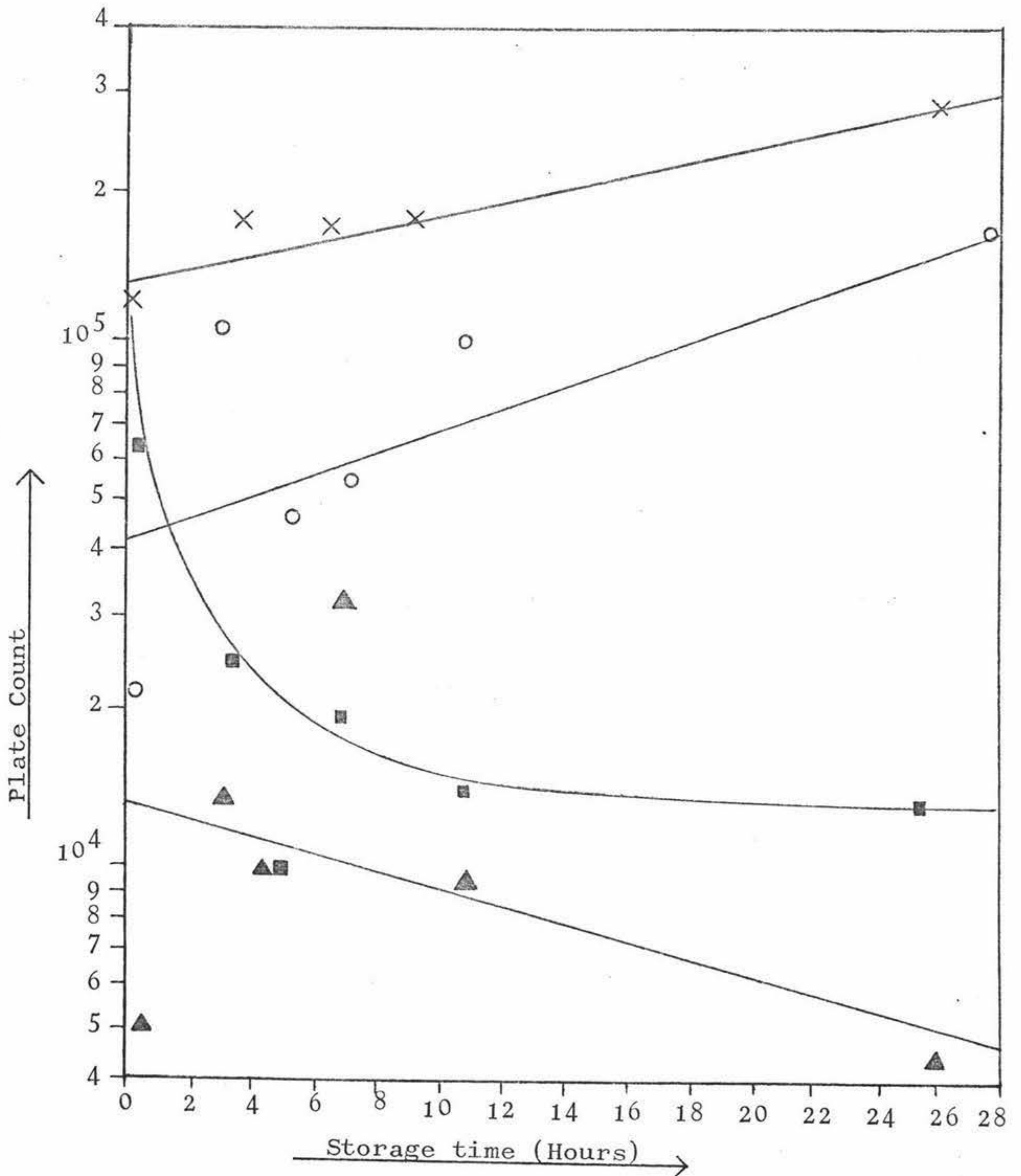


FIGURE 5:5 Bacterial growth curves from raw fish materials: Staphylococcus Counts

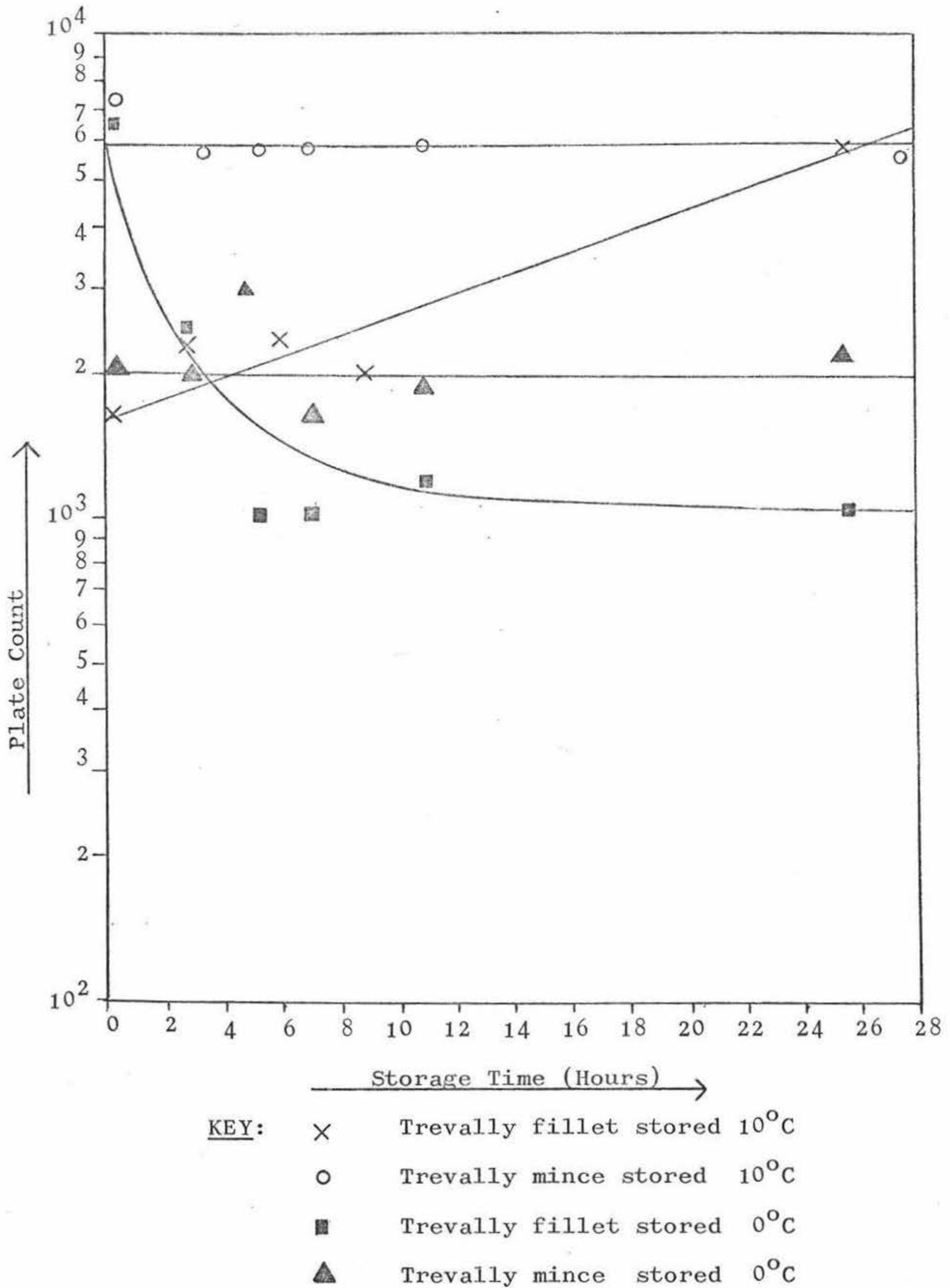


FIGURE 5:6 Bacterial growth curves from raw fish materials: Coliform Count

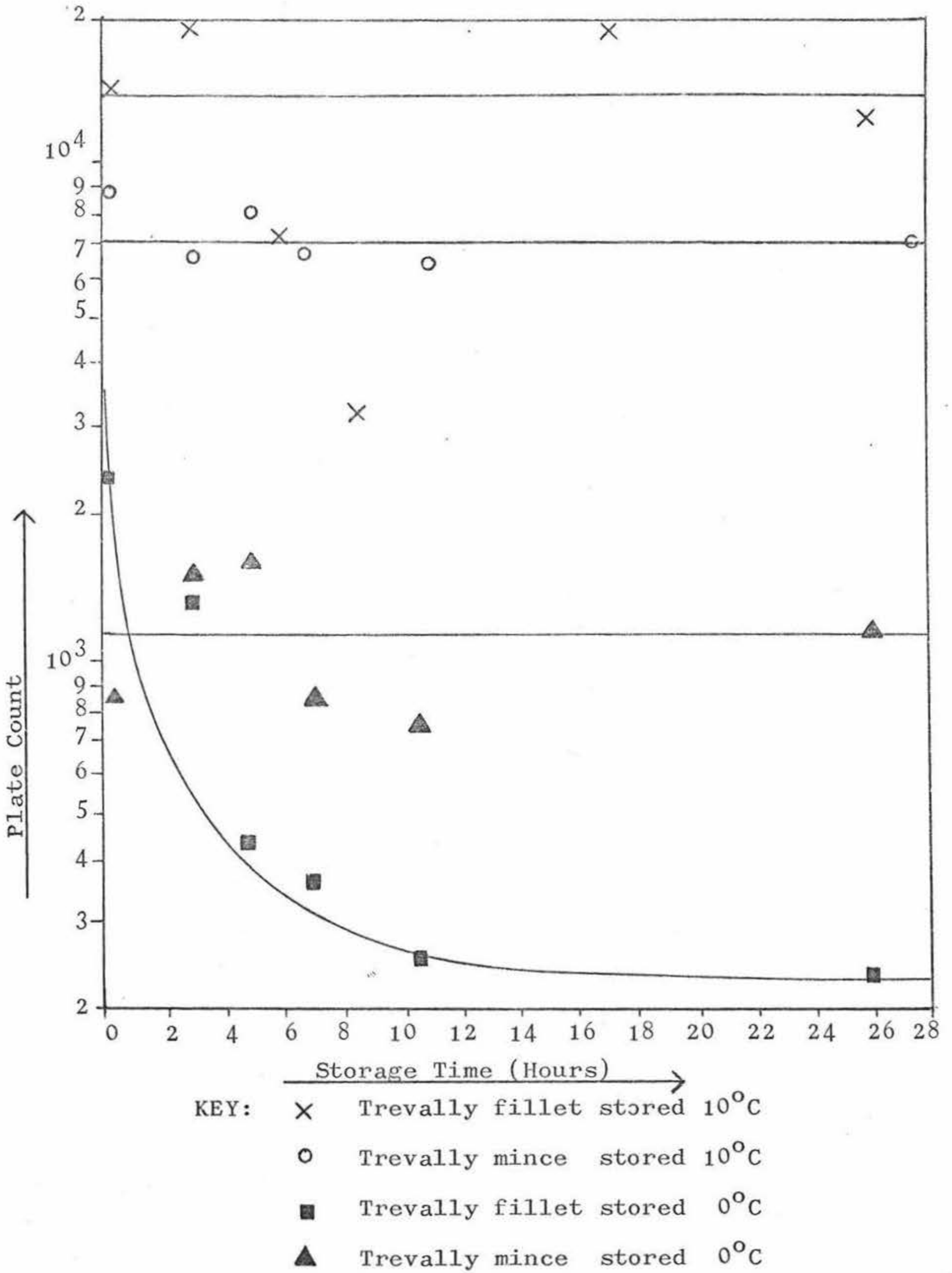


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TABLE 5:2 Bacterial numbers after cooking

	Number of organisms per gram
Total count ($25^{o}C$)	57
Total count ($10^{o}C$)	nil
Staphylococci	4
Coliforms	nil

Organoleptic quality of the raw materials

The samples of the fish examined were found to be of variable quality. The odour of the fillets prior to cooking was faintly fishy, but not objectionable. The flesh colour varied from one fish to another. Some fillets still had a tinge of pink colour, while others were discoloured, showing marked brown to yellowish colour. The flesh was firm to touch, but not elastic.

The cooked sample had a strong fishy smell which was objectionable. In addition, the fish tasted slightly rancid. The colour of the cooked sample was off-white, tending to be grey, with the darker flesh a very dark, greyish or reddish brown.

Discussion of the Results

Quality of the raw material

The discolouration of the flesh, and the unpleasant odour and flavour of the cooked fish does indicate that the fish used in this work was not of good quality. This feature is borne out by the bacteriological tests and will be discussed further in the sections which follow.

Other tests carried out at various times in the fish factory show that quite a large proportion of the fish processed during the season is likely to be of a similar quality.

Total counts of fish mince processed from snapper frames during December 1972 varied between 10^5 and 10^6 organisms per gram. Coliforms on the same samples varied between 10^2 and 10^4 organisms per gram.

Sumner and Wilson (1973) during a bacteriological survey of fish at various stages of processing, examined frozen/thawed gurnard, trevally and barracautta. Their results, shown in Table 5:3, are of a similar order to the results summarized in Table 5:1.

TABLE 5:3 Bacteriological quality of frozen/thawed fish (from Sumner and Wilson 1973)

	Number of organisms per gram		
	Gurnard	Trevally	Barracautta
Total count (25^oC)	8.0×10^5	1.8×10^5	1.0×10^6
Coliforms	4.4×10^3	1.4×10^3	5.3×10^3
Staphylococci	3.3×10^2	2.0×10^1	2.2×10^2

Bacteriological Growth Rates in Products Tested

(a) Growth curves in products stored at 0^oC

The growth curves in the raw materials stored at 0^oC (Figures 5:3 - 5:6) did not show significant change during the twenty four hours immediately after thawing, and bacterial numbers appeared to be kept in check, both in the fish mince and in the fillet.

The apparent reduction of bacterial numbers from the trevally fillet during the first ten hours of storage is probably due to the loss of moisture from the surface of the fillet. The fillet was stored on an open tray, and the effect of the low humidity in the chiller was observed to cause the surface of the fillet to become dry. In industry the fillets are stored in 40 lb containers, and loss of moisture from the surface of the product would not affect the bulk of the product and so the observed reduction in bacterial numbers is unlikely to occur in commercial practice.

(b) <u>Growth curves in product stored at 10°C</u>

Bacterial numbers increased slightly at 10°C but not significantly during the twenty four hours immediately after thawing.

In earlier work on mince prepared from fresh snapper (Sorensen 1972) it was found that bacterial numbers increased from 8×10^5 to 6×10^6 within four hours at 10°C, that is, traversing one log phase every four and a half hours. The apparently slower growth rate observed in the current experiment may be due to a proportion only of the bacteria being in their log phase after freezing and thawing.

Due to the discrepancy between the earlier work and the present work, the results are not conclusive, and the storage of freshly prepared mince is not recommended. For the same reason, more work in this field will have to be done before safe storage and working temperatures can be established.

Effect of heating the product

The heating process, primarily used to gel the soluble fish protein and thereby effect the restructuring of the product as described in Section 2, is also effective in reducing bacterial numbers in the product. The effectiveness of the heating process (Figure 5:1) can be seen by comparing the bacterial numbers in the cooked (Table 5:2) and the uncooked (Table 5:1) product.

The total counts in the cooked product are, in the main, due to bacterial spores and vegetative cells of thermoduric bacteria which have survived the heat treatment. Provided the product is cooled and frozen immediately after the heating operation, it is unlikely that these organisms will cause further spoilage of the restructured product.

More important, however, is the survival of staphylococci in the product. With a pH of 6.7, a salt content of 2% and the absence of large numbers of competitive organisms, the product is an excellent medium for the growth of staphylococci that were not destroyed during heating or which are present as a result of recontamination. For this reason strict temperature control and plant hygiene must be closely adhered to after the cooking of the product is completed.

Recontamination of the cooked product is likely to be most difficult to control, and equipment such as band saws and the batter and crumbing machine are suspect in this regard. Ingredients used in the batter and crumb application will also need to be checked regularly, and standards will have to be prepared for the batter, to ensure the final product does not

contain an unacceptable number of bacteria. Packaging materials may also become dirty and contaminated, and to avoid contamination these should be stored in a clean and dry area separate from the processing factory. Close control of sanitation procedures will be necessary and a code of practice for sanitation will need to be prepared.

Quality Standards

Quality which has been lost through faulty handling of fish at sea and in the factory cannot be restored by any form of processing. Only low grade food can be expected from poor quality raw material. In the case of a new development, such as the restructured fish product, the use of substandard quality fish could jeopardize the development. For this reason there is a need to set quality standards on the fish used in the process, such standards being set to ensure both the safety and acceptability of the product.

As there are no bacterial standards enforced for seafoods, it is necessary to use recommended standards as a guide.

Shewan (1970) has revised standards recommended for seafoods. Although some of these standards vary widely, there appears to be a general agreement that the total count (25^oC) for seafoods should not exceed $1 \times 10^5/g$. This figure has been put forward as a tentative standard by Codex Alimentarius and in practice has been found a reasonable standard to expect of fish processed under hygienic conditions (Neufeld 1971).

Coliform standards are recommended to ensure that the product has been processed under sanitary conditions.

Recommended standards vary between a general coliform count and a faecal coliform test based on <u>Escherichia coli</u>. The reason for this is that some authorities (Neufeld (1971) claim that coliforms in general cannot be used to differentiate between fisheries products manufactured under good and unsatisfactory sanitary conditions, whereas there can be no reasonable explanation other than insanitation for the presence of high levels of faecal coliforms. An advisory standard of 230/100 g is used for faecal coliform counts in Canada.

Other authorities, claiming coliforms to be effective "indicator organisms" for faecal contamination, have proposed standards requiring coliform levels of less than 100/g for frozen products, while some authorities have set standards of not more than 100/g for precooked products. The tentative coliform standard suggested by the Codex Alimentarius is less than 200/g (Shewan 1970).

Standards for coagulase-positive staphylococci are a useful means of controlling poor handling and processing practices. In addition, these organisms must be considered potentially enterotoxigenic which under certain conditions, can cause food poisoning. Most standards set levels which aim to use the staphylococcus count as a means of controlling poor handling technique, and coagulase-positive staphylococci are generally required to be less than 100/g. An exception to this is the Canadian standard, in which a count of greater than 1000/g is cause for rejection, and a count of greater than 100/g is used to alert the laboratory to more intensive sampling and to instigate the necessary remedial action in the plant.

Although staphylococci are potentially enterotoxigenic, most authorities agree that appreciable levels of the enterotoxin are produced only after a considerable growth of the organisms, usually a population of at least millions per gram must be obtained (Frazier 1967). Even so, enterotoxin production is a factor that must be considered since under certain conditions (especially between 21 and 31°C) the enterotoxin may become evident within 4 to 6 hours. Of further concern in this study is the heat stability of the enterotoxin, which can withstand boiling for 20 to 60 minutes. Thus the heat treatment proposed for the restructuring process would not destroy the enterotoxins, should any be present.

The tentative bacteriological standard for fish to be used for manufacturing the restructured product is summarised in Table 5:4. This standard is based on the above discussion and is considered to be practically attainable and is one that can be determined using fairly simple procedures. As the hygiene of the plant is upgraded it may be possible, or even prove necessary, to make certain aspects of the proposed standard more rigid.

TABLE 5:4 Standard proposed for fish to be used for the production of the restructured product

	Count not to exceed
Total Count (25°C)	1×10^5/g
Coliforms	200/g
Coagulase-positive staphylococci	1000/g

Areas for further investigation

It is evident from the result of this examination, and from work done at other times, that much of the fish processed would not meet the standards proposed in Table 5:4. Sumner and Wilson (1973) reported that at two stages of processing fish were found to be particularly susceptible to bacterial growth:

(1) When frozen fish were thawed in large tanks of water overnight the bacterial count was found to increase by one log phase.

(2) Total counts increased by three log phases during battering and crumbing operations. During this time the product which had previously been cooked, was also recontaminated with both staphylococci and coliforms.

In addition it was found that the plant was frequently not properly cleaned after a day's production, and as a consequence the fresh product was contaminated from this source the following day.

The work reported indicates the need for a regular quality control and inspection programme. The object of such a programme would be to ensure that an acceptable standard of raw material quality, product handling and of plant hygiene and cleanliness be maintained so that spoilage of the fish and the product can be controlled.

In the following discussion sources of contamination which occur in fish processing are briefly outlined as a basis

for future work.

The flora of living fish depends upon the microbial content of the waters in which they live. The slime that covers the outer surface of fish has been found to contain bacteria of the genera Pseudomonas, Achromobacter, Micrococcus, Flavobacterium Corynebacterium, Sarcina, Seratia, Vibrio, and Bacillus (Frazier 1967). This initial contamination can be reduced considerably by thorough washing.

In addition to the flora naturally present on the outer slime of the fish, contamination can arise from mud scraped off the sea bed by the net. The bottom mud may contain large numbers of bacteria, including pathogens. Clostridium botulinum type E has been isolated from the bottom mud in various parts of the world, including U.S.A., Europe and Asia, and strains of Salmonella have been isolated from mud samples taken in New Zealand. Many fishing vessels in New Zealand are not fitted out with proper washing facilities, and there is a tendency to wash the fish in the dumping locker. This area is sometimes very restricted, and washing the fish in the dumping locker may spread mud and contamination on to fresh ice and throughout the working area of the hold.

Most fish landed in Auckland is caught off the Northland coast. These waters are known to reach temperatures in excess of 24°C during the height of the summer season. At these temperatures bacteria can multiply rapidly, and the temperature of the fish must be lowered as quickly as possible if serious contamination is to be avoided. Flaked ice is normally used to reduce the temperature of the fish, but is

only effective if it is distributed evenly around the fish as they are stored away.

During the early stage of spoilage, most bacteria will be confined to the skin, gills and the gut. This is generally true for up to seven or eight days storage if the fish have been correctly iced. Thus the bacterial numbers on the fish can be reduced quite considerably by thorough washing when the fish are unloaded at the factory.

If the fish have been roughly handled at sea, such as by an excessively long haul in a trawl, or by incorrect use of dividing boards and tools such as fish picks and shovels, they may be bruised or crushed to the point where the skin has broken or the gut has rupture. In such cases the bacteria will invade the flesh and contamination will become deepseated. Such fish will be unlikely to meet the standards outlined (Table 5:4) and washing will not be effective in reducing the bacterial numbers.

The effectiveness of washing depends on how this operation is performed. Continuous systems in which high pressure water is sprayed over the surface of the fish can be most effective, whereas batch systems in which fish are dumped in a large tank of water can in fact be a source of contamination.

Washing, however effective, will not remove all the bacteria on the fish, and the remaining bacteria will continue to multiply after washing. The rate at which these bacteria multiply will be dependent on the temperature of the fish, and if spoilage is to be controlled, it is important that the temperature of the fish is not allowed to rise greatly during

processing. In this regard some authorities have proposed a maximum temperature of 7.5°C. As water used in some Auckland fish processing factories commonly exceeds 21°C during summer, it may prove necessary to incorporate a water chilling plant in the factory.

Containers, processing equipment, benches and tools which come in contact with the fish during handling and processing are also known to spread infection. Likewise airborne dust from dirty surroundings may blow on to and contaminate the product.

To control these course of contamination a thorough cleaning routine is essential. Fish slime, whilst easy to remove when wet, is very difficult to remove once it has been allowed to dry. Fish residues are also more easily removed when wet. It is therefore important that containers, benches and processing equipment are cleaned immediately after use. In addition the plant should be washed and scrubbed down at least daily. Due to the tenacious nature of any dried fish slime, it is necessary to make use of an abrasive cleaner such as sandsoap.

Processing plant, such as the meat/bone separator and mixing machines in which the product is spread over the working surface of the machines, should be stripped down and washed during the tea breaks and in the lunch hour. This will avoid this equipment building up a high bacterial load over a period of time thus increasingly contaminating the fresh product.

While bacteria are prevented from entering the flesh of the fish, and are contained on the surface of the fish, spoilage is most readily controlled. When the fish is passed through the meat/bone separator, the pressing and shearing actions spread the bacteria from the surface of the fish, throughout the mince as it is produced. For this reason the production of a large stockpile of fish mince should be avoided, and the mince should be processed only at the rate at which it can be put into a more stable form. Thus the fish mince production should be geared to the requirements of the restructuring process, the fish being stored in the whole or gutted state rather than as mince.

Provisional cleaning Schedule

The cleaning schedule for the restructuring process presented here is a guide line, the purpose of which is to provide the processor with suggestions that will help to produce a product having an acceptable microbiological quality.

Daily Cleaning Sequence

Dismantle Processing equipment.

Mechanically or manually remove loose dirt by scraping and brushing equipment and floors.

Rinse all equipment and floors thoroughly with cold or warm (less than $40^{o}C$) water. Because fish residues and other proteins coagulate at higher temperatures and may become baked onto the contact surface, remove these materials at temperatures below $40^{o}C$ ($100^{o}F$) early in the cleaning process.

Scrub down either by hand or with high pressure hose systems with a warm (45-50°C) solution of an acceptable detergent or scouring agent made up to suppliers instructions.

Rinse twice with clean warm water at a temperature of about 60°C (140°F). Hot water is more effective than cold water in rinsing off fats, oils and inorganic material, but too hot water at this stage may lead to build up of residues that are not effectively rinsed away.

When equipment and floors have been washed, scrub open drains and empty sump baskets.

Sanitize all equipment with an acceptable bactericidal agent, chlorine compounds are most widely used, and recommended strengths are given in Table 5:5.

Rinse with cold potable water (a thorough rinse with cold potable water should follow any operation involving a chemical sanitizing agent).

Repeat the santizing process immediately before start up.

TABLE 5:5 Concentrations of Chlorine recommended for use in fish processing plants (cited Perry Lane 1970)

Use	Chlorine available (ppm)
Wash water	2 - 100
Rinse water on hands	100
Clean smooth surface (wash basins, urinals, glassware)	50 - 300
Clean smooth wood, metal or synthetic surfaces (new boxes, new table tops, conveyor belts, machines)	300 - 500
Rough surfaces (worn tables, old boxes, concrete floors and walls)	1000 - 5000

In addition to the daily cleaning operation outlined above, some of the plant may be required to be dismantled and washed down at regular intervals throughout the day. A schedule for these operations is summarised in Table 5:6.

Note: No work was done on the cleaning requirements of the batter and crumb machine. The dismantling and cleaning of the batter pumps and holding tank are based on published work, and may have to be modified in a specific situation.

TABLE 5:6 Cleaning requirements of processing plant in production

Unit	Cleaning Operation	
	Regular or Hourly Intervals	Tea and Lunch breaks
Fish container	Thoroughly rinse and wash immediately after they are used.	
Meat/bone Separator		Remove drum and wash down drum and belt
Mincing and Mixing machines	Dismantle at end of each run and wash parts thoroughly	
Extruder and extrusion nozzles		Dismantle and wash down
Continuous cooker and cooling conveyor		Clear of product and wash down
Cutting machines		Dismantle and wash down
Batter and Crumb machine		Dismantle pump, and wash down; discard old batter
Packing Table		Clear of product and wipe down

SECTION VI

Process and Plant developed for the production of restructured fish mince

The restructured, or heat-gelled fish mince, has been developed for use in the production of battered and crumbed products, such as fish fingers, thus for this section the restructuring process is considered in the overall production of these products.

Fish fingers are traditionally processed from skinned and boned fillets. The fillets are packed and frozen in metal trays or moulds, and the fingers are cut from the frozen blocks. It is important that the fingers are not allowed to thaw after they are cut, as they depend on their frozen state to retain their shape. The cut portions are passed through a battering and crumbing plant, from which they are sometimes deep dried to stabilize the structure of the product and improve its appearance. Packaging is followed by refreezing the product which is stored at $-18^{o}C$.

Raw fish mince is viscous and tacky after beating in the presence of salt. This property allows the mince to be extruded in the form of a continuous band. A further characteristic of the restructured product is that it does not depend upon freezing to retain its shape, but upon the gel which is formed upon cooking. These properties have been used as a means to simplify the traditional fish finger process, and enable recovered fish mince to be used in a continuous process which is described in this Section.

Process outline

The procedure used for the preparation of test samples

TABLE 6:1 Operation sequence used for the preparation of test samples of restructured fish mince and the process used for the production of product for use in fish finger production

Operation sequence in test sample preparation				Operation sequence in continuous process			
No.	Operation	Materials handling, storage, deliberate delay	Sampling measurement & inspection	No.	Operation	Materials handling, storage, deliberate delay	Sampling measurement & inspection
1		Receive fish mince		1		Receive fish mince	
2	Grind mince to appropriate particle size			2	Grind mince to appropriate particle size		
3			Weigh mince & additives	3			Weigh mince & additives
4	Mix in additives solubilizing myofibrillar protein			4	Mix in additives solubilizing myofibrillar protein		
5		Hold 24 hours for salt equilibration		5		Load mixture into extruder	
6		Pack mixture into a cooking vessel		6	Extrude as a continuous band		
7	Steam 20 min. at 100°C			7	Steam for 9 min at 100°C		
8	Cool to 30°C			8	Cool to 30°C		
9			Allow to equilibrate 12 hours to 0°C				
10	Cut test sample			9	Cut into fish finger size portions		

of restructured fish mince outlined in Section III can be divided into ten operations. These operations have been modified as shown in Table 6:1, to enable the restructured mince to be produced in the form of a continuous band of product. The product can be cut into fish finger portions, and battered and crumbed as it is produced.

The two holding stages in which the salt was allowed to equilibrate and the temperature of the product brought to 0°C have been eliminated from the continuous process to allow for the direct handling of the product from one operation to the next, and to avoid hold ups in production. This change necessitated modifications to the process as discussed later.

Development of pilot plant

In the pilot plant the beaten mince was extruded as a band 0.5 in. thick by 6 in. wide, using a sausage filler fitted with a specially fabricated extrusion nozzle. The extruded fish product was collected on a conveyor which carried it through a steam tunnel in which the product was cooked to set it in its gel form. After cooking, the product was lifted off the belt using a scraper blade and collected on trays for cooling. A schematic diagram of the pilot plant is given in Figure 6:1, and a plant specification is summarised in Table 6:2.

FIGURE 6:1 Schematic diagram of the pilot scale plant used in experiments on the development of a continuous process for the production of restructured fish mince

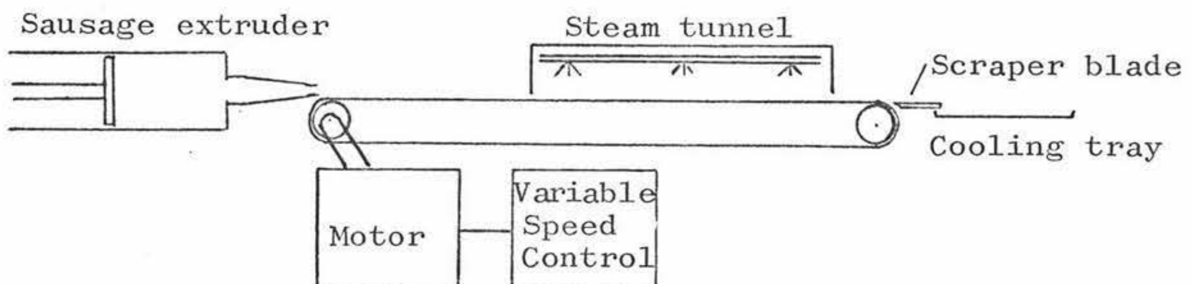


TABLE 6:2 Pilot Plant Specification

Item	Sausage filler fitted with extrusion nozzle	Conveyor belt	Steam tunnel
Function	Extrude raw beaten mince into a continuous band 0.5 in. thick, 6 in. wide	Carry mince through steam tunnel	Cook mince to effect the gellation of the product
Size	Inches diameter, inches long	9 in. wide 7 ft 6 in. long	4 ft 6 in. long
Capacity	10 lb product	13.5 - 120 lb/hour	40 lb/hour
Material of construction	Body - mild steel Nozzle - stainless steel	Frame: Dexian Belt: Plastic coated plied canvas	Stainless steel
Temperature range	- $10^{\circ}C$	$10^{\circ}C$ - $100^{\circ}C$	$100^{\circ}C$
Motive Power	Manual	1 h.p. DC motor with variable power input	.25 lb steam/lb product

Discussions on Experimental Work and developments on the pilot plant

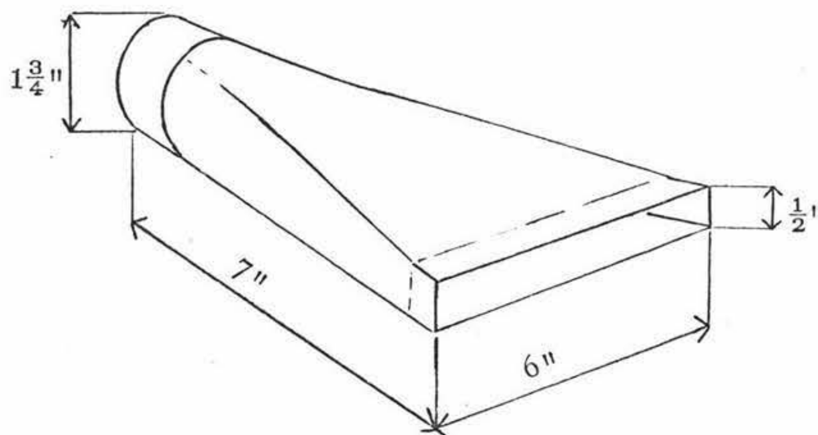
1. Extrusion

In early experiments the mince was placed in a vertical, bottomless hopper situated directly over the conveyor. The hopper was fitted with an adjustable opening through which the mince was drawn by the action of the conveyor belt. This operation was not successful as the product did not flow uniformly and it was found that positive displacement was required to extrude the mince uniformly.

Sausage fillers are used for the extrusion of meat and fish products into casings, and such a unit was readily applied to the extrusion of the restructured product.

The sausage filler used in the pilot plant was a manually operated model in which the product was forced out of the cylindrical holding vessel by the action of a piston. The filler had a capacity of 10 lb product, and was fitted with a specially fabricated stainless steel nozzle to extrude the fish in the form of a band 0.5 in. thick and 6 in. wide. A detailed diagram of this nozzle is shown in Figure 6:2.

FIGURE 6:2 Detailed drawing of nozzle used for the extrusion of continuous band of restructured fish 0.5 in. thick by 6 in. wide



Effect of salt concentration and aperture size on the operation of the extruder

If the fish mince was filled directly into the sausage filler and extruded, extrusion was not uniform and the band was incomplete, containing holes and gaps, making it unsuitable for the production of fish fingers. When salt is beaten into the mince with a high speed mixer the product becomes viscous and extrusion is more uniform.

When the extrusion nozzle shown in Figure 6:2 was used, the extruded band was consistent and uniform when 0.8% salt was beaten into gurnard mince. Work was also done on a similar nozzle giving a band 0.25 inches thick. This nozzle was found to give a consistent band of product when only 0.6% salt was added, suggesting extrusion is more uniform on the narrower nozzle.

It was also found that as the rate of extrusion was increased so the extruded product became more uniform. An explanation for these observations is that the extruded product becomes more uniform as pressure across the extrusion nozzle is increased. In the above cases this was achieved by an increase in the product viscosity, decreasing the nozzle aperture and by increasing the rate of extrusion.

This suggests that the design of the nozzle will affect the uniformity of extrusion, a longer nozzle in which the pressures are higher giving the more uniform extrusion. As work was not done on a great variety of extrusion nozzles, this is an area requiring further investigation.

Tests on multi-aperture nozzles

A multi-aperture nozzle was tested for the direct extrusion of fish fingers. The aim of this investigation was to eliminate one of the cutting operations that would be required if a single band of fish were to be cut into fish fingers. The nozzle tested contained six apertures, each aperture extruding a band of fish 13/16 in. wide by ½ in. thick. It was found that extrusion was not uniform, some apertures blocking, causing others to extrude extra fish. This problem was not overcome and the multi-nozzle extruder was not considered practicable.

2. Heat gellation of continuous band of product

In the pilot plant the cooking operation took place as the product was carried through a steaming tunnel on an endless conveyor belt.

The conveyor was driven through a series of pulleys and a 75:1 reduction box by a 1 h.p. DC motor. The motor was connected to a variable power source, which allowed for belt speeds of 2 to 18 inches per minute.

The steaming tunnel was constructed of stainless steel in the form of an enclosed box 4 ft 6 in. long, 10 in. wide and 6 in. high. The conveyor belt was made to run along the bottom of the steaming tunnel, ensuring good support for the product on a flat surface. This reduced the tendency for the gel to crack or to break apart as it was formed. The ends of the tunnel were closed by flexible rubber flaps, which reduced steam losses, but allowed the product to pass through the

tunnel. Steam was fed directly into the tunnel by a 1 inch sparge pipe running down its length. Six 3/16 in. holes were drilled at 8 inch intervals in the top of the pipe to give even steam distribution in the tunnel.

A polyvinylchloride coated plied canvas belt was used to convey the product through the steam tunnel. Although the manufacturer claimed that the belt could withstand steam cleaning, it was recommended that normal operating temperatures should not exceed 71°C.

Evaluation of optimum cooking time

When the mince is run into the steaming tunnel for cooking, heating is effected by the steam injected over the top of the product. Heat is transferred from the top to the bottom of the extruded band of mince, thus the point of slowest heating is at the interface between the product and the conveyor belt.

The temperature at the interface between the product and the conveyor was measured by taping a thermocouple wire to the conveyor belt and extruding the product over the probe. The cooking time was changed by varying the belt speed and appropriate adjustment of the extruding rate. The temperature at the point of slowest heating for a variety of cooking times is shown in Table 6:3. The approximate equilibration temperatures (i.e. temperature mid point of slab at the end of the steaming tunnel) are also shown.

TABLE 6:3 Relation between the cooking time and the temperature of the restructured fish product

Conveyor speed (in./min.)	Cooking time (min.)	Temperature at interface between product and conveyor (°C)	Approximate equilibration temperature (°C)
18	3.0	26.0	47.0
10	5.4	46.5	72.5
7	7.5	54.0	79.5
6	9.0	62.6	86.0
4	13.5	74.4	90.0

It was found that the band of product did not gel throughout until the interfacial temperature exceeded 53°C. After cooking to this temperature the product was seen to crack or break apart. This tendency was reduced as the temperature was increased, and when the interfacial temperature was allowed to reach 65°C breaking up occurred only at lower salt levels.

Whilst it is desirable to cook the product to higher temperatures for the reduction of bacterial numbers, it can be seen that small increases in cooking temperatures require an increasingly long time due to the decreasing temperature difference between the steam and the product. A long cooking time requires a longer cooking tunnel for a given capacity, or would result in a reduced capacity for a given tunnel.

Optimum binding of the product appeared to have occurred when the interfacial temperature reached 65°C. The coinciding equilibration temperature was measured at about 75°C, which from Section V, can be seen to give effective pasteurization. From Table 6:3 it can be seen that these temperatures

are achieved after cooking the product in the steam tunnel for nine minutes.

3. Effect of binding character of the restructured product on the operation of the plant

In Section III it was shown that the textural characteristics of the restructured product could be modified by varying the salt content, the moisture content and by grinding the product to change the mince particle size. It was also shown that in order to have similar textural qualities to those of cooked fillet, the product was required to have a high Degree of Breakage and to rupture under low pressures. These characteristics are achieved when the product has relatively weak binding properties. When such products were passed through the pilot plant the band broke apart as it was scraped off the conveyor. This effect could be reduced by increasing the salt level in the product and not adding any water. Although these products were readily processed, they were dry and tough on eating.

The restructured fish mince processed on the pilot plant appeared more loosely bound than a similar mixture cooked in a glass petri dish. This may be due to the effect of condensate collecting on the product in the steaming tunnel, diluting or weakening some of the structure before it is gelled.

A further consideration on the pilot plant is that the product is to be removed while it is still hot. In this condition some of the gel-forming material has not set and the gel structure is not as strong in the warm product as when it is chilled.

A large number of fish mince mixtures were investigated before a product was made with good binding properties and an acceptable eating quality. The properties of some of these mixtures are summarised in Table 6:4. Extrusion in this table refers to extruding the product through the 0.5 in. nozzle shown in Figure 6:2, and the product was cooked for 9 minutes in the steam tunnel.

From Table 6:4 it can be seen that an acceptable compromise in the handling properties and the eating quality of the product was not possible from a single treatment. The problem was eventually overcome by dividing the mince into two portions, and treating each portion separately.

One portion was regarded as the binder and was prepared by beating or grinding the mince in the presence of added salt and water until the mixture became tacky and showed good binding characteristics. The second portion of the mince was not treated in any way, allowing the particles of mince to retain their natural structural qualities.

A number of restructured products based on the principle of mixing an untreated portion of mince in a binding fraction were prepared. The preparation and the properties of the two most successful products have been summarised in Table 6:4. The raw blend was generally found to have properties similar to the binding phase, in that the mixture was very sticky and extruded uniformly.

The handling properties of the cooked product were found to depend on the properties of the binding phase and on

TABLE 6:4 Summary of properties of different fish mince preparations processed on the pilot plant

Ingredients	Preparation	Processing properties		Eating Quality
		Raw mixture	Cooked product	
100.0% Gurnard mince	Beat in Kenwood 90 seconds	Poor extrusion	crumbled apart easily	Dry, crumbly
99.5% Gurnard mince 0.5% Salt	Beat in Kenwood 90 seconds	Poor extrusion	crumbled apart easily	good
99 % Gurnard mince 1 % Salt	Beat in Kenwood 90 seconds	Good extrusion	good binding, handled well during process	rubbery & chewy
87 % Gurnard mince 12 % Water 1 % Salt	Beat in Kenwood 90 seconds	Poor extrusion	crumbled apart easily	very good
65.6% Gurnard mince - untreated 34.4% Binder consisting of: 85.0 % Gurnard mince 16.36% Water 1.64% Salt	Grind binder in bowl chopper for 5 min. Gently mix binder into untreated mince.	Good extrusion	Some crumbliness	very good
50.0% Gurnard mince - untreated 50.0% Binder as above	Grind binder in bowl chopper for 5 min. Gently mix binder into untreated mince	Very good extrusion	Good handling	very good

the ratio of binding material to untreated material, while the eating quality appeared to be dependent on the presence of untreated fish mince.

The sample made from 50% binding material and 50% untreated mince (Table 6:4) was found to have very good processing properties, as it extruded uniformly and did not break apart when handled after cooking. In addition, the product is considered to have very good eating qualities and the texture resembled that of cooked fish. This can be seen in Table 6:5, where the results of the textural characteristics of the restructured product evaluated on the OTMS equipment have been compared with the textural qualities of cooked fish fillet.

TABLE 6:5 Comparison between the textural characteristics of restructured fish mince processed from 50% binding material and 50% untreated material, and cooked trevally fillet

Classification	Mean Value	
	Trevally Fillet	Restructured Gurnard
Youngs Modulus	0.3 lb/in.^{2}	0.206 lb/in.^{2}
Average Break Force	0.5 lb	1.47 lb
Degree of Breakage	30.0 %	24.5 %

Cooling Requirements

It was found that even though the product held together in the form of a wide band when cooked, there was a tendency for the product to break if it was cut into fish finger sized portions hot. If, after cooking, the product was

chilled to $0^{o}C$ it was very readily cut without any signs of crumbling or breaking. The reason for this is thought to be due to the gel forming materials not setting completely until the product has reached cooler temperatures.

Chilling to $0^{o}C$ requires the product to be held for about 12 hours in the chiller. Such an operation would break the continuous nature of the process, and requires extra handling of the material. A compromise was therefore made to find a temperature at which the product could be cut efficiently after cooling on a continuous system at ambient temperature.

It was found that the product of 50% binder, and 50% untreated mince, could be cut and handled as fish finger sized portions when the product was cooled below $30^{o}C$. The cooling rate of the 0.5 in. restructured fish slab was measured by inserting a thermocouple into its centre with the thermocouple connected to a Varian millivolt recorder. The cooling experiments were carried out on open mesh trays. In one set of experiments a fan was used to agitate the air over the product. The results of the cooling experiments, which are summarised in Table 6:6, are based on cooling in air at a temperature of $21^{o}C$.

Cooling the product to below $30^{o}C$ does have the advantage of enabling more gel forming material to set. It is also noted that $30^{o}C$ is the optimum growth temperature for many bacteria (Section V), and so if the product is cut, battered and crumbed at this temperature, quality standards will have to be particularly stringent.

TABLE 6:6 Comparison between cooling a slab of 0.5 in. restructured fish mince on an open mesh tray by natural convection and by air circulated with a fan - air temperature 21°C

Time (min.)	Temperature in centre of product	
	Cooled by natural convection (°C)	Cooled by circulated air (°C)
0	75.0	75.0
2	70.0	60.5
4	59.5	47.5
6	51.6	37.5
8	43.5	32.0
10	37.2	27.8
12	32.8	25.5
14	30.0	23.8

The disadvantage of cooling the product to below 30°C is that it takes an increasingly long time. If the product is to be continuously cooled on a conveyor, it is important that the cooling time be kept to a minimum, as the length of the cooling conveyor is directly related to the required cooling time. In view of this situation it was decided not to cool the product below 30°C.

Experimentally (Figure 6:6), it was found that the centre of an 0.5 inch slab of restructured fish mince took 13 to 14 min. to cool from 75°C to 30°C by natural convection. Cooling time was shown to be reduced to 9 min. if a blower fan was used to circulate the air over the product.

Cutting the product

After the restructured product has been cooled to 30°C or less, it is firm and can readily be cut with a sharp knife or wire cutter.

The slabs of restructured fish mince prepared on the pilot plant were cut into fish finger sized portions using a kitchen knife and a metal guide. A continuous cutting system was not investigated.

Battering and crumbing of restructured mince

Work was not done on developing a batter for the restructured product. It is noted however, that in the traditional fish finger process some of the batter pickup is a result of the batter freezing on to the frozen product. As the restructured product is not frozen prior to battering, it may be necessary to re-formulate the batter used on the frozen product to allow for this feature.

Preparation of restructured product with layers simulating myotome structure of fish muscle

An alternative to the continuous process is the production of the restructured fish mince as blocks of layered product.

The layering effect is achieved by extruding the mince into a band 0.20 inches thick and gelling the product by passing it through the steam tunnel as described previously. The band is then lifted off the conveyor and collected in an oscillating container; the oscillating movement causes the band to fill the box in layers. The product is then pressed to

remove air pockets between the layers and then frozen.

After freezing, the blocks are cut so that the layers of the restructured product simulate the myotome organisation in a natural fillet. The product is then battered and crumbed in the usual manner. While the extra operations involved in the layering process will add to the operation costs of the process, the features of the layered product may be found to increase the value of the product to offset these costs.

SECTION VII

Manufacturing plant and cost analysis of an industrial process to produce fish fingers from restructured mince

Note:

1. The manufacturing operation outlined in this section has been designed for the specific needs of Jaybel Nichimo Limited Auckland.

2. Although the plant specified is based on the work outlined in the previous section on the pilot plant, it is recommended that a qualified engineer is consulted to study and advise on the proposed process design.

3. The cost analysis shown at the end of this section was prepared in December 1973, and costs may have to be reassessed if the process is considered at a later date.

Process capacity

The Company aims to produce 1,000,000 lb of fish fingers per annum. As the product can be produced during the winter months from stocks of frozen fish, it is assumed that the process will be operated independently of the day to day catch. This would allow the factory to operate for 2,000 hours per annum on standard time, further increases being possible through overtime.

The plant has been designed for food handling and quick cleaning, so that 30 minutes a day should be adequate for the clean up operation outlined in Section V. The size of the process would allow it to be stopped for lunch breaks, although it is recommended that morning and afternoon tea breaks

are staggered to allow for continuous operation. It is also recommended that a supervisor and an assistant be appointed to prepare materials and plant 30 minutes prior to normal starting time. These steps would enable the plant to be operated for about $7\frac{1}{2}$ hours during a normal 8 hour day, that is, about 1870 hours per annum.

From these figures it can be shown that the plant is required to have the capacity to produce 540 lb finished product per hour.

Process mass balance

The manufacturing plan for fish fingers using restructured mince is derived from the work done on the pilot plant, and the operation sequence follows the continuous process summarised in Table 6:1. The raw materials used in these operations are fish, salt and water. Batter and bread crumbs are then applied to complete the product.

The quantity of material used in the process has been evaluated on the basis of information gained in industry and on the experimental developments outlined in this thesis. The process mass balance shown in Table 7:1 is a summary of the materials used in the production of 540 lb saleable product.

A slight gain in weight experienced during cooking, due to the collection of condensate on the surface of the product, and the loss of weight during cooling are not noted in the mass balance as these effects compensate for each other.

In addition to the usual loss of material which occurs during production, a loss factor of 5% of the finished

product has been allowed for. This factor will cover the effective loss of saleable material which occurs due to over weight packs and product damaged after production.

TABLE 7:1 Process mass balance of materials required for the production of 540 lb fish fingers from restructured fish mince

Operation	Materials used in process	Materials cut for further processing	End Products
Heading and gutting	795 lb Gurnard	554 lb headed and gutted Gurnard	241 lb fish heads & offal
Meat/bone separation	554 lb headed and gutted Gurnard	414 lb fish mince	140 lb scrap
Grind and beat binding fraction	186 lb mince 36.3 lb water 3.7 lb salt	228 lb binding fraction	
Mix untreated mince into binder	228 lb binder 228 lb mince	456 lb mixed product	
Extrude cook and cool	456 lb mixed product	456 lb heat gelled product	
Cut into fish fingers	456 lb heat gelled product	425 lb fish fingers	31 lb cutting losses
Batter fish fingers	425 lb fingers 73 lb batter	487 lb battered product	11 lb batter losses
Crumb fish fingers	487 lb battered fish fingers 88 lb crumbs		12 lb crumb losses 24 lb product losses 539 lb finished product

Selection and design of manufacturing plant

The pilot plant described in section VI was used as the determining principle in the selection of manufacturing plant for the production of fish fingers from restructured mince. As the Company already owns equipment which can be used in this process, new equipment has been specified only where no alternatives are available. Where possible standard processing equipment has been specified, although specially fabricated plant is required for the cooking and cooling operations.

The plant selected for the manufacturing process is outlined in the following list:

1. Heading and gutting

The fish will be headed and gutted by skilled knife hands using standard methods. Automatic processing plant is available to handle a variety of fish species, but as the process is not directly connected to the restructuring operations or developed experimentally in this thesis, it will not be discussed here.

2. Meat/bone separation

An Ikenchi Fish Separator with a capacity of up to 1500 lb recovered mince per hour is available for the meat/bone separation of the fish material. This unit recovers the edible flesh by a combination of tearing and shearing actions, forcing the flesh through a perforated drum. The flesh recovered from this machine was used in the experimental work, with good results and will be used in the manufacturing process.

3. Grinding

Although a bowl chopper was most satisfactory in the experimental work, grinding has also been found to be effective using a mincer fitted with a 4 mm plate. As the Company owns such a unit it is not proposed to purchase a bowl chopper.

The use of the mincing machine for the grinding operation will require the grinding and mixing operations outlined in Table 6:1 to be modified. Instead of grinding the mince in the presence of salt and water, as in the bowl chopper, the use of the mincer requires the mince to be ground separately. The ground mince can then be beaten in a high speed mixer with the salt and water to form the binding fraction. The mixture is finally completed by mixing in untreated mince at slow speed.

4. Mixing

A Yanagiya mixing machine used in the factory for the preparation of fish cake mixtures has a capacity of 250 lb product. This machine can be used at both high speed and low speed, making it suitable for the mixing required in the restructuring process.

5. Extrusion

The success of the sausage filler for the extrusion of the fish mixture in the experimental work and the availability of these machines as standard items for the processing industry, has lead to the selection of a sausage filler for the manufacturing process. The ideal machine would have a holding capacity of about 250 lb product. In addition, the machine should have independent fixtures for three extrusion nozzles. A suitable machine is not currently available on the New Zealand market.

Three companies dealing in food processing plant are agents for sausage fillers and could import a suitable machine as required. It is recommended that the following companies be consulted to offer specifications on their respective machines.

Henry Berry Limited	Auckland
R.C. Dimock Limited	Auckland
A.M. Satterthwaite & Company Ltd	Auckland

6. Extrusion nozzle

The experimental extrusion nozzle was fabricated to extrude the fish into a band 6 inches wide and 0.5 inches thick. In the production process it is necessary to extrude the band 36 inches wide. It is likely that the extrusion of a uniform band of this width will be difficult to achieve with a single extruder, and so it is proposed to extrude three bands 12 inches wide adjacent to one another. To ensure uniform extrusion each nozzle will be required to be fitted to an independent filling head from the machine.

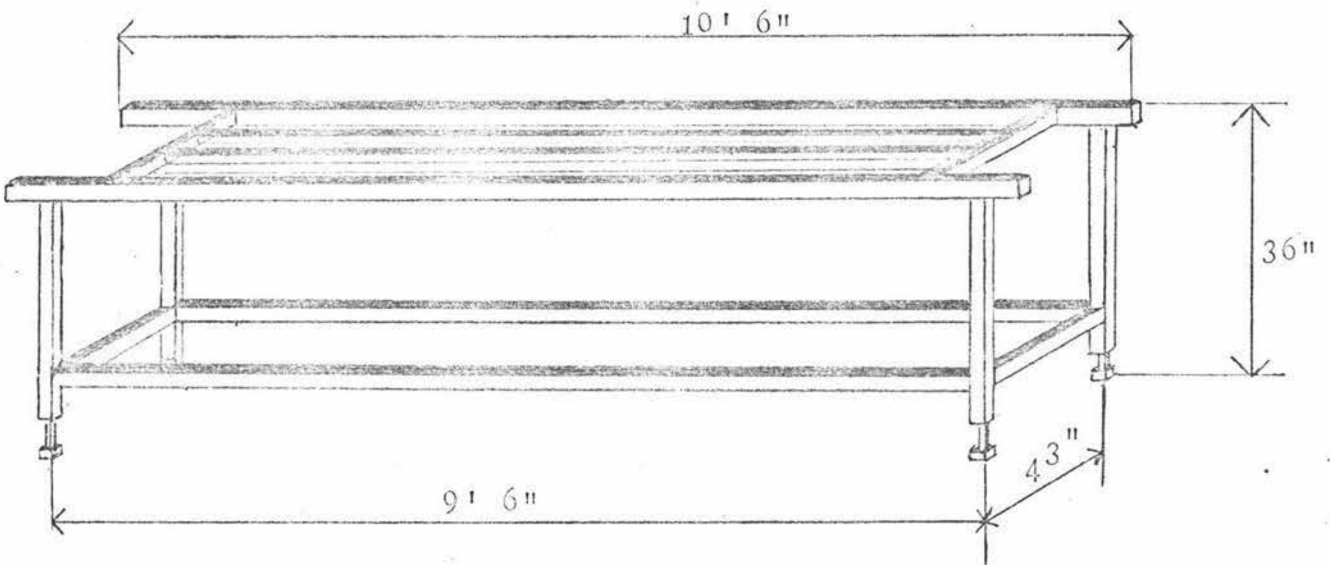
The nozzles are to be fabricated from 20 gauge stainless steel.

7. Steaming tunnel

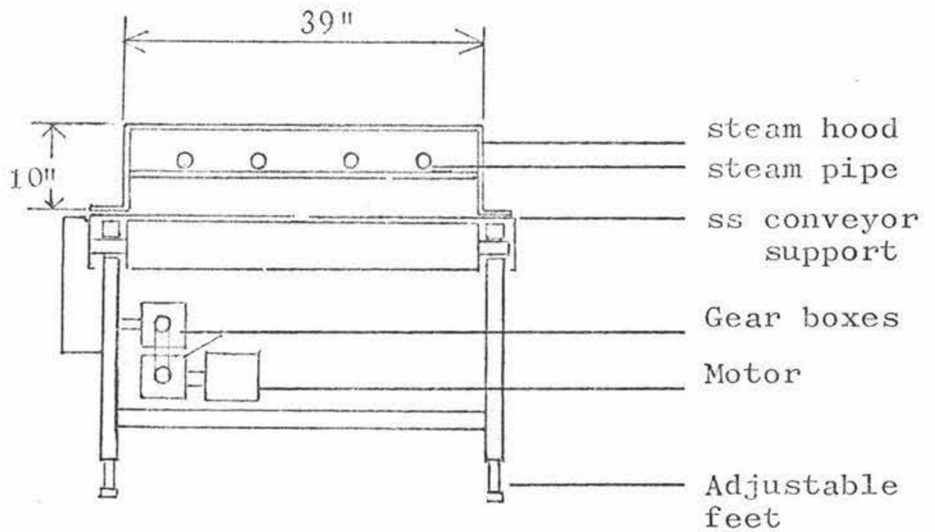
The cooking process developed in the pilot plant requires the product to be steamed for 9 minutes on a continuous conveyor as it passes through a steam tunnel operated at atmospheric pressure (100°C). From the process mass balance shown in Figure 7:1 it can be seen that 456 lb product is to be cooked each hour in the manufacturing process. Thus it can be shown that the conveyor is required to hold 68.3 lb (1.09 ft^3) product in the conveyor when in operation.

FIGURE 7:1 Details of proposed steam tunnel to cook 446 lb restructured fish product per hour (Scale ½ in. = 1 ft)

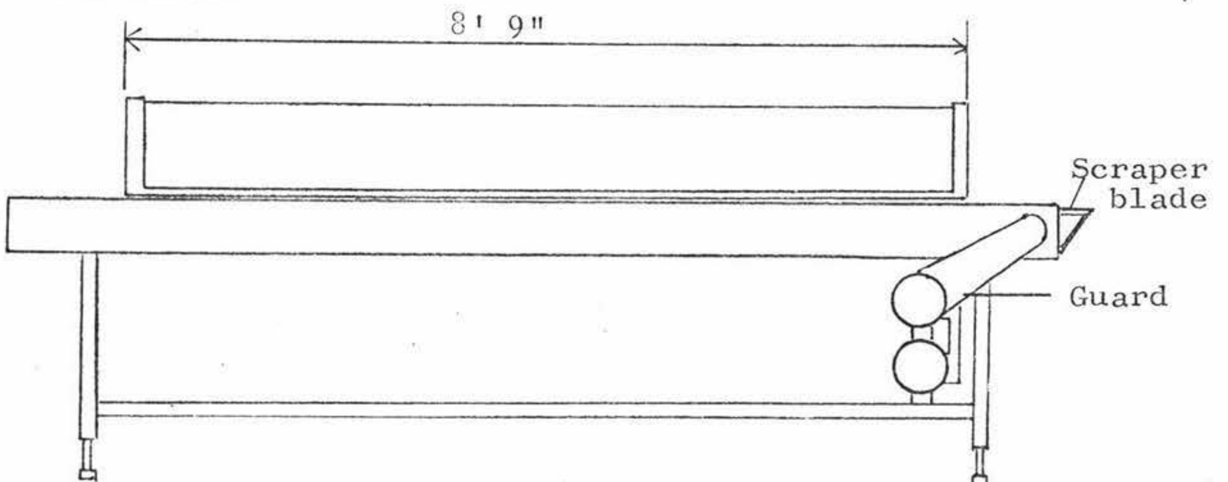
1. Frame Detail



2. End View



3. Side View



If the product is extruded on to a standard 36 inch conveyor as a band 0.5 inches thick, the conveyor will carry 7.85 lb (0.125 ft^3) product per foot length; that is, the required 68.3 lb product can be carried on 8 ft 9 inches of Conveyor. Allowing 1 ft for the inspection of the extruded fish mince before steaming and 9 inches after steaming for the removal of the product, it can be seen that the conveyor is required to be 10 ft 6 inches long. In addition it can be shown that the conveyor is to be operated at 0.972 ft per minute (11.7 inches per minute), the 9 minute cooking cycle being achieved as the product passes through the steam tunnel 8 ft 9 inches long.

A plan of the proposed steam tunnel is shown in Figure 7:1. The construction specifications of the steam tunnel as well as the cooling conveyor have been included as a basis for cost estimating only, and may be modified by a qualified engineer should more suitable materials become available.

Construction specification of steam tunnel

Frame:	Construct of 2 inch RHS.128 mild steel; Galvanise by hot dip process.
Steam hood:	Construct of 16 gauge aluminium, support with 2 x $\frac{1}{4}$ inch aluminium bars.
Conveyor Support:	Construct of 20 gauge stainless steel
Steam Lines:	Steam distributed by 1 inch steam pipe
Conveyor belt:	Anziel Stedar PVC Food Quality conveyor belting: Code: TS 140 No. of Plys: 2 Max. working tension per inch width: 140 lb Covers: Top 1/32 Bottom skim

Cover material: PVC

Overall thickness: 0.155 inches

Minimum pulley diameter: 6 inches

Temperature range: $-10^{o}F$ to $+160^{o}F$

Friction value: 0.60

Note: Although this conveyor is only certified for a normal working temperature of up to $160^{o}F$ ($71.2^{o}C$) it was considered to be the most suitable material available in New Zealand. Provided the plant is operated with a 0.5 inch band of product, the conveyor belt temperature should not exceed $67^{o}C$. The conveyor is resistant to steam cleaning.

Drums: 6 inch diameter x 38 inch face;
$1\frac{1}{4}$ inch shaft.

Rollers: 2 x 36 inch Polykleen Low Temperature Gravity Roller

Drive: Motor 1 h.p.
Gear Box: 1 x 60:1 4 inch reduction box
1 x 35:1 4 inch reduction box

The hood of the steam tunnel is to be constructed so that it can be lifted off the conveyor for cleaning. Steam is to be distributed in the steamer by four 1 inch pipes; the top of each pipe drilled with 16 x $\frac{1}{8}$ inch holes at 6 inch centres.

8. Cooling conveyor

The cooling operation has been developed to be completed on an open mesh conveyor, collecting the cooked product directly from the steam tunnel. As the cooling operation follows on after the product has been cooked, the cooling conveyor must be operated at the same speed as the cooker, that

is 0.972 ft per minute (11.7 inches per minute).

The time required to cool the product will vary with the ambient temperature, but in view of the results of the experimental work on the pilot plant, should not exceed 9 minutes. This time will require the cooling conveyor to be 9 feet long, and 3 feet wide.

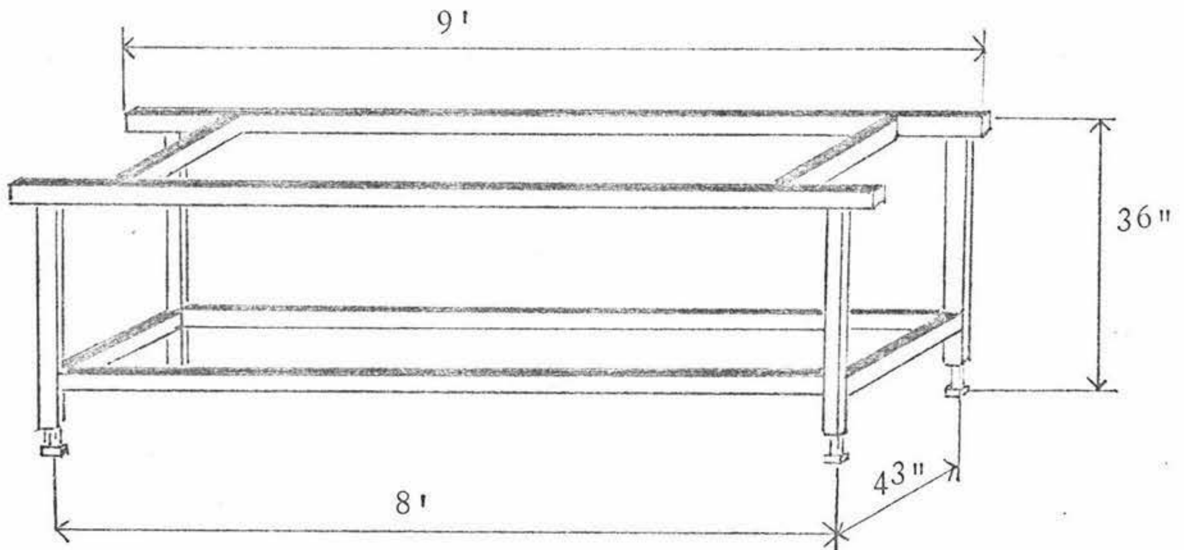
A detailed drawing of the proposed cooling conveyor is shown in Figure 7:2. The height of this plant as shown in the diagram may have to be adjusted to allow the product to be dropped on to the receiving belt of the batter and crumb machine after cutting.

Cooling conveyor specification

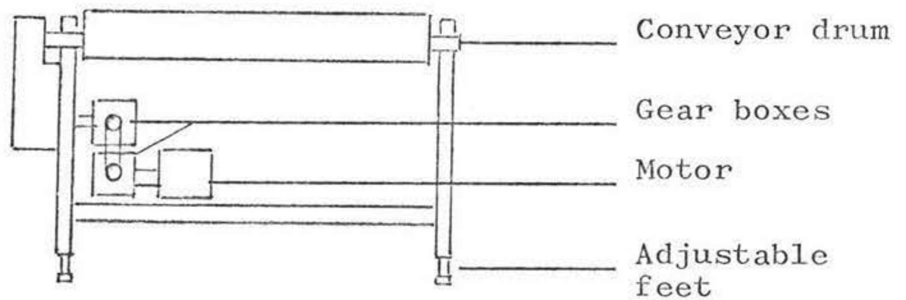
Frame:	Construct of 2 inch RHS.128 mild steel; galvanised by hot dip process.
Conveyor belt:	Stainless steel mesh belt, type FC 712
	Gauge: 16
	Mesh size: Pitch $\frac{1}{2}$ inch
	Spiral size $\frac{1}{2}$ inch
	Construction: Balanced weave, alternating left and right scroll with crimped pin.
	Support: 3 x 36 inch Polykleen Low Temperature Gravity Rollers.
Drums:	6 inch diameter x 38 inch face,
	$1\frac{1}{4}$ inch shaft;
	driving drum to be rubber coated.
Drive:	Motor: 1 h.p.
	Gear box: 1 x 60:1 4 inch reduction box
	1 x 35:1 4 inch reduction box

FIGURE 7:2 Details of proposed cooling conveyor to cool 446 lb restructured fish product per hour (Scale $\frac{1}{2}$ in = 1 ft)

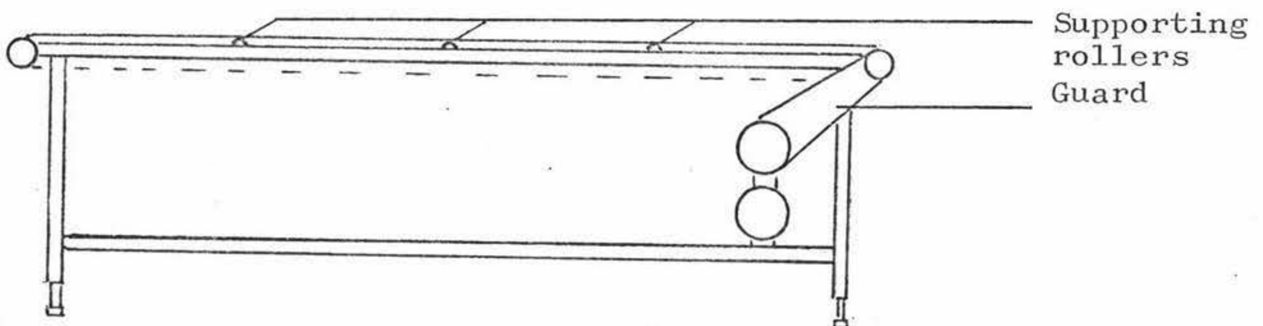
1. Frame Detail



2. End View



3. Side View



Cooling fans: $\frac{1}{2}$ h.p. motor

4 blade flexifan fitting

9. Cutting plant

Although a continuous cutting sytem for the restructured product has not been investigated, it is envisaged that either static, oscillating, or circulating knives, or wire cutters, or a combination of these can achieve the desired results.

It is recommended that research on the use of these systems be completed before the overall restructuring process is implemented. Experimental work on the cutting systems can be done in the factory on restructured mince prepared by cooking the product in trays.

In the cost estimate which follows, it has been assumed that oscillating knives will fulfill the cutting requirements, and that the development and fabrication of a suitable plant can be achieved for $1500.00.

Estimated cost of processing plant

The cost of the processing plant required for the manufacture of fish fingers produced from restructured mince is summarised in Table 7:2.

Existing plant has been included in this estimate to be used in the calculation of manufacturing costs, and has been valued at the current replacement cost less depreciation.

The cost of new plant has been estimated from information forwarded by agents and suppliers as shown.

TABLE 7:2 Cost estimate of Processing Plant used for the manufacture of fish fingers from restructured mince

Process Plant Specification	Item Cost $	Plant Cost $	Source of Information
Yanagiya Fish Separator		3664.00	Existing plant: current replacement costs less depreciation
Yanagiya Fish Chopper		964.00	
Yanagiya Mixing Mill		1410.00	
Ataka Battering & Crumbing Machine		5716.00	
Packing Table		650.00	
Wrapping Table		500.00	
Total value of existing plant		12904.00	
Oil-hydraulic sausage filler		2500.00	R.C. Dimock Ltd
Extrusion Nozzles		105.00	E. Edwards Estimator
Steamer:			
Motor and gear boxes	750.00		Paykel Bros Ltd
Drums	142.00		
Polykleen Rollers	8.00		
PVC Conveyor belt	80.00		
Vee belt guard	150.00		E. Edwards Estimator
103 ft x 2 in. RHS.128 mild steel	50.00		
11 x 5 ft^2 20 gauge s.s.	105.00		
10 x 5 ft^2 16 gauge aluminium	30.00		
20 ft x 2 in. x $\frac{1}{4}$ aluminium bar	34.00		
32 ft x 1 in. steam pipe	13.00		
12 ft x $\frac{1}{2}$ in. s.s. rod	6.00		
Galvanising charge	60.00		
Fabrication charge	730.00		
		2158.00	
Cooling conveyor:			
Motor and gear boxes	750.00		Paykel Bros Ltd
Drums	222.00		
Polykleen rollers	12.00		
S.S. Mesh belt	231.00		Faulkner & Collins
72 ft x 2 in. RHS.128 mild steel	36.00		E. Edwards Estimator
Vee belt guard	150.00		
Galvanising charge	50.00		
Fabrication charge	750.00		
2 x $\frac{1}{2}$ h.p. fans	172.00		
		2201.00	
Cutting machine:		1500.00	
Total cost of plant to be purchased		8464.00	

Building and Structures

The manufacturing process is to be installed in an existing building valued at $320,000. The building is sited on leasehold land, half the annual lease of $7,800 being accounted to production.

As the process will occupy about 25% of the available floor space in the building, this figure has been used in costs relating to the building and structures.

Installation of Services

The factory is supplied with all the services required, including refrigeration, electricity, water, steam, compressed air and drainage, and installation of services is included in the building costs.

Direct Manufacturing costs

1. Raw Material Costs

The raw materials used in the production of one million pounds of saleable fish fingers have been evaluated in Table 7:3.

TABLE 7:3 Cost of raw materials used in the production of 1,000,000 lb fish fingers

Material	Requirement (lb)	Unit value $	Total Value $
Green Gurnard	1,475,000	0.09	130,750.00
Salt	6,840	0.0366	250.00
Batter (Flour	63,100	0.0597	3,760.00
Batter (Cornflour	18,000	0.1617	2,905.00
Bread crumbs	163,000	0.18	29,394.00
Total cost Raw Materials			166,859.00

2. Labour

The labour requirements summarised in Table 7:4 have been evaluated on the basis of the production of 540 lb product per hour.

TABLE 7:4 Summary of Labour requirements for the production of 540 lb fish fingers from restructured mince

Operation	Labour requirement
Heading and gutting	2 men
Washing fish and meat/bone separation	1 man
Grinding mixing and extrusion	1 man
Battering and crumbing	1 man
Packing	6 women

Current labour rate:

men	$1.39 per hour
women	$1.13 per hour

Costs have been allowed for two men to spend one hour a day on plant start up and cleaning down operations. It is assumed that the factory will work 8 hour days, 250 days per annum.

The annual labour rate has been evaluated as follows:

Standard hours of work:

5 men at $1.39 for 2,000 hours	$13,900.00
6 women at $1.13 for 2,000 hours	$13,560.00

Overtime:

2 men at $2.09 for 250 hours	$ 1,045.00
Annual payroll	$28,505.00
Holiday pay	$ 1,140.00
Allowances: 5 men: 48c/week	$ 120.00

6 women: 46c/week	$ 138.00
Annual direct labour cost	$29,903.00

3. Supervision

The process is under supervision of one qualified supervisor, who will have the responsibility of preparing batter mixes and assisting in weighing out additives such as salt and water. In addition, the supervisor will be responsible to the quality control department for the quality of the product and for plant hygiene.

A salary of $4,500 per annum has been allowed for the Supervisor.

4. Maintenance

Maintenance costs have been estimated as 5% of the value of plant, and is based on the cost estimation of Aires and Newton (1967) and Buchanan and Sinclair (1966).

Estimated maintenance cost = 5% of $21,368.00
= $1,069.00 per annum.

5. Utilities

(a) Refrigeration

Refrigeration is costed against the product at 2c per lb; this is a standard value used by the Company.

Estimated cost of refrigerating 1,000,000 lb
product at 2 c per lb = $20,000 per annum

(b) Electric Power

The power consumption of the process is evaluated in Table 7:5, and is based on the power rating of individual units

of plant.

TABLE 7:5 Evaluation of electric power consumption

Unit	Power Requirement (K Watt)	Daily Operation (hours)	Daily power usage (K Watt hrs)
Meat/bone separator	1.50	4	3.00
Mince	0.75	2	1.50
Mixer	1.50	4	6.00
Sausage filler	0.375	7.5	2.81
Cooking conveyor	0.75	7.5	5.63
Cooling conveyor	0.75	7.5	5.63
Cooling fans	0.75	7.5	5.63
Batterer and crumber	1.12	7.5	8.40
Packing line	2.75	7.5	20.60

Total daily electric power requirements = 59.20 K Watt hours

Assuming a 250 day year;

Annual power requirement is 16,800 Kilowatt hours

Current cost for industrial power is 1.7c per unit

Annual power costs are $284.00.

(c) Steam

Approximately one quarter pound of steam is used in the cooking process for every pound of product processed.

Steam is costed at $15.0 per 1000 lb

Annual usage = 250,000 lb

Annual steam cost = $375.00

(d) Water

Water is used throughout the factory for cleaning, refrigeration, washing of fish, etc. and the amount of water

used directly in the process would not effect this greatly. The water costs have therefore been evaluated as 25% of the water used at present.

Present water rates: $8,000.00 per annum

Cost of water accounted to process is $2,000 per annum

(e) Drainage levy

A drainage levy of $100 is paid each month for effluent discharge from the two factories operated by the Company. Assuming each factory pays an equal levy, the drainage accounted to the restructuring process is 25% of $50 per month.

Annual drainage levy: $150.00

Summary of utility costs

	$
Refrigeration	20,000
Electric power	284
Steam	375
Water	2,000
Drainage	150
	22,809

6. Packing Materials

Fish fingers are currently sold in three packs. The sales distribution of the product is known, and it is assumed that sales will continue in these same proportions.

The packaging requirements and their costs have been evaluated on a basis of the expected sales, and are shown in Table 7:6. A wastage factory of 2% is allowed for in the estimate, the cost of the outer has been included in the unit cost.

TABLE 7:6 Requirements and cost of packaging materials for the distribution of 1,000,000 lb fish fingers

Pack	Proportion of current sales	Expected Sales (lb)	Packs Required (no.)	Unit Cost ($/1000)	Total Cost $
3 lb	30%	300,000	102,000	22.70	2,310
16 oz	20%	200,000	204,000	53.25	10,850
10 oz	50%	500,000	816,000	62.50	51,060
Total cost of Packaging Materials					$64,200

Indirect costs

The indirect costs of payroll overhead are covered by a charge of 5 c per lb product sold. This is a standard costing procedure used by the Company, and also covers distribution charges.

Total indirect cost at 5 c per lb of product sold = $50,000.00

Fixed costs

1. Depreciation

The building, valued at $322,000 is depreciated at 4% per annum. As the plant occupies 25% of the available floor space in the building, the depreciation to be acounted for by the restructuring process is $3,220.00.

Processing equipment is depreciated at 15%. The value of the processing plant is $21,368.00; giving a depreciation of $3,200 for the first year of production.

Total depreciation for buildings and processing equipment is $6,420.00.

2. Lease of property

The building occupies approximately half the area of the leased land. Thus the lease accounted for in this process is 12.75% of $7,800 per annum; that is, the lease accounted for equals $975.00.

3. Insurance

Insurance figures were not obtained but were estimated at 1% of capital cost:

Insurance costs:	Building	$805.00
	Equipment	$214.00
Total insurance		$1019.00

Selling price

The selling price of the fish fingers depends upon the size of the pack. The wholesale price of the three packs and the total price of the product sold has been evaluated in Table 7:7.

TABLE 7:7 Estimated wholesale selling price and sales of the fish fingers in the three standard packs

Pack	Unit Price ($)	Estimated Sales (No.)	Total Sales ($)
3 lb	1.80	100,000	180,000
16 oz	0.64	200,000	128,000
10 oz	0.44	800,000	352,000
Total annual sales			660,000

The selling price has been evaluated on the assumption that the restructured product will continue to sell at the same price as is obtained for the product prepared from fish fillet. If the selling price is to be reduced the gross profit shown below will have to be modified.

It is also noted that the estimate has been prepared on the basis of Gurnard being used in the process. The use of other species of fish and seasonal variations in price will affect the manufacturing costs and thus require their modification.

Profit and Loss account for the proposed process

	$	$	$
Sales			660,000
Manufacturing costs:			
Direct costs:			
Raw materials	166,859		
Labour	29,903		
Supervision	4,500		
Maintenance	1,069		
Utilities	22,809		
Packaging Materials	64,200		
		289,340	
Indirect costs:			
Fixed costs:			
Depreciation	6,420		
Lease	975		
Insurance	1,019		
		7,314	
Gross profit		313,346	
Totals		660,000	660,000

Discussion

Much of the work on the pilot plant was carried out with frozen gurnard mince. The mince had been specially recovered by the meat/bone separator in Auckland, frozen, and airfreighted to Palmerston North. The fish were of good quality, and the restructured product prepared from the mince had excellent quality characteristics.

Barracautta, which is not popular due to its bone structure, could be successfully used in the restructuring process. Care should be taken in removing the kidney and blood line along the back of the belly cavity when this fish is used. The use of worm infested fish is to be avoided.

A great deal of work was done to adapt trevally to the process. The fish has a good flavour, and restructures well if it is of good quality. The main problem in using trevally mince is due to the colour of the cooked product, which was found to be unacceptable. Some work was done to try to make the product acceptable by the use of additives such as nicotinic acid and sodium nitrites to give the cooked mince a faint pink colour such as is associated with salmon. The use of additives was not investigated fully, and it is recommended that a full research project be undertaken to study the use and the safety of such additives in fish products.

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