THE UNIVERSITY OF MANITOBA

DESIGN AND FABRICATION OF A PROTOTYPE APPARATUS FOR THE FORMATION OF PROTEIN FIBRES

BY

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF FOOD SCIENCE

WINNIPEG, MANITOBA

APRIL 1987

ISBN 0-315-37229-X

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ABSTRACT

A prototype apparatus was designed and fabricated to successfully form protein fibres on a continuous pilot-scale basis. The fibres were formed using a protein extract prepared from freshwater whitefish (Coregonus clupeaformis). The apparatus consisted of a Moyno Pump Assembly and a Fibre Conveying Device. The Moyno Pump Assembly was comprised of a Moyno cavity pump equipped with a 40 - nozzle extruding die, capable of forming forty fibres simultaneously and at a rate of 30 mm/second. The Fibre Conveying Device consisted of a wire belt which remained submerged while travelling through a coagulating medium - this device permitted the formed fibres to coagulate on a continuous basis. An acetified ethanol-based medium was used as the coagulant.

Various operating parameters appeared to reduce the strength of the formed fibres. These included exposure of the fibres to water, an acidic coagulating medium (ie. pH 4.5 to 6.0), and low ethanol content of the coagulating medium (ie. less than 70%). Maximum fibre strength occurred when the fibres were not exposed to water, the pH of the coagulating medium was maintained within the range of 6.5 to 7.3 and the ethanol content of the coagulating medium held within the range of 80 to 95%. Preliminary investigations have also shown that it was possible to simulate the textural properties (ie. bite) of various

seafood items by exposing prepared formed fibre bundles to specific thermal processes.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitute to many individuals, without whose help and cooperation, would not have made this project possible. My heart felt appreciation goes to Dr. M.A.H. Ismond for her seemingly endless hours of support and constructive criticism and to Dr. E.D. Murray for his guidance and faith. Sincere thanks are extended to Dr. G.E. Timbers and staff at E.S.R.C. for their dedicated assistance during the design and fabrication phase of this project and to the Structures and Mechanization department for their excellent draftsmanship in preparing the schematic drawings. Special appreciation is extended to Dr. M. Kalab and Paula Allan-Wojtas of the Food Research Centre for their hours spent on the microscopic analysis, George Morris of the Analytical Services division, Paul Stephen for his computer-related assistance and to Larry Barker for his understanding. The financial support for this project, offered by the Department of Fisheries and Oceans as well as by the Department of Food Science, was also greatly appreciated.

I remain eternally grateful to my family and to Donna, for enduring through this sometimes endless and often thankless time. I am especially grateful to my mother and father for their never ending love and support - who sometimes felt they were last on my list, but always remained first in my heart.

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1. INTRODUCTION

Food texturization has added a new dimension in the production of engineered or fabricated foods. It is well recognized that much of the pleasure of eating is derived from the textural qualities and the significant variety of differences in texture of our natural foods (Hartman, 1977). Considerable study has been directed toward the measurement and control of the texture of natural foods. Since both the microstructure and macrostructure of natural foods are so important, and since texture contributes significantly to food acceptance, it is important in fabricated foods to utilize or develop new processes which give the desired qualities and which increase the acceptance of the fabricated product.

Of particular interest at this time is the fabrication of various seafood analogues from surimi-based materials. Surimi is a Japanese term for mechanically deboned fish flesh that has been washed with water and mixed with cryoprotectants to insure a lengthy frozen shelf life (Lee, 1984). It is used as an intermediate product for a variety of fabricated seafoods, such as crab legs and flakes, shrimp, scallops and lobster. Although surimi may be produced from most fish species, the primary species used is the Alaskan pollock. Due to the tremendous stock of this underutilized species, large quantities are available at

relatively low cost. For this reason, most surimi-based seafood analogues are relatively inexpensive to produce and are therefore very competitive in the consumer market.

Despite the large stock of Alaskan pollock, there is growing attention being paid to the utilization of large supplies of freshwater fish species available in central Canada. In particular, the inland lake and water streams within Manitoba contain a tremendous supply of various fish species. Several examples of these species include whitefish (Coregonus clupeaformis), mullet (Catostomus commersoni) and tullibee (Coregonus artedii). According to the Manitoba Freshwater Fish Marketing Corporation, approximately 7 million kilograms of whitefish are harvested in Manitoba on an average annual basis. Of this total, approximately 6 million kilograms are of grade A, (export quality) which are primarily exported to various European and American markets. The remaining 1 million kilograms, comprised of lower quality, parasitized cutter-grade fish, is deboned and exported to various American markets for use in dumplings, sausages, etc.

The food industry has applied various food texturization techniques to produce a variety of products such as breakfast cereals, pasta products and restructured meats. However, very little work has been carried out on the texturization of surimi-based materials. The major problem associated with the fabrication of surimi-based seafood analogues is the lack of available processing technology (Lee, 1984). In particular, the lack of a

fibrous character was found to detract mostly from the acceptability of the fabricated analogues presently available on the market (Martin, 1975).

At present, crab flavoured pollock sections (ie. Harbour House Kamaboko) represent the most successful attempt at producing a texturized seafood product. The extent of the texturization process involves forming thin sheets of a surimi-based material, scoring the sheets into thin strips and rolling them into a round bundle to simulate the texture of crab legs (Nolan, 1983). Unfortunately, this product provides only a very limited degree of fibrous texture and mouthfeel. Although the consumer demand for this product has steadily risen during the past several years, considerable improvement in the fibre texturization process is still required before greater acceptance occurs.

Since there existed considerable room for the innovation and development of a method to simulate the texture of various seafood products, a study was undertaken by the Food Science Department at the University of Manitoba to develop such a process. The study would entail the design and fabrication of a fibre forming apparatus that would have the capability of continuously producing fibres using a surimi-based material. The ultimate goal was to use the fabricated fibres in combination with a suitable base material (surimi) with the hope of simulating the textural properties of various seafood products. In

order to carry out this objective, the operating principles of existing texturization techniques (ie. screw extruder and spinnerette) were researched to determine which priniciples could be applied towards the conceptual design of a proposed fibre forming apparatus.

2. REVIEW OF LITERATURE

Most food texturizing operations employ either a spinnerette or screw extruder device to produce various fabricated foods. Unfortunately, the screw extruder as well as the spinnerette have had only limited success in the texturization of surimi-based seafood analogues. Although a modified use for the screw extruder has been incorporated in the production of crab flavoured pollock sections, it appeared the texturization process in this application was not carried out by the action of the extruder but rather by the action of a scoring device after the material had travelled through the extruder (Lee, 1984). This process is in contrast to most typical applications whereby the texturization process is carried out while travelling through the extruder.

On the other hand, spinnerettes have been used on a bench-scale basis to fabricate actual fibres from various materials including surimi. It was later demonstrated by Young and Olsen (1974) that the fabricated fibres impart a fibrous texture, similar to that of various seafood products, when combined with suitable base materials.

2.1. TEXTURIZATION THROUGH SPINNING

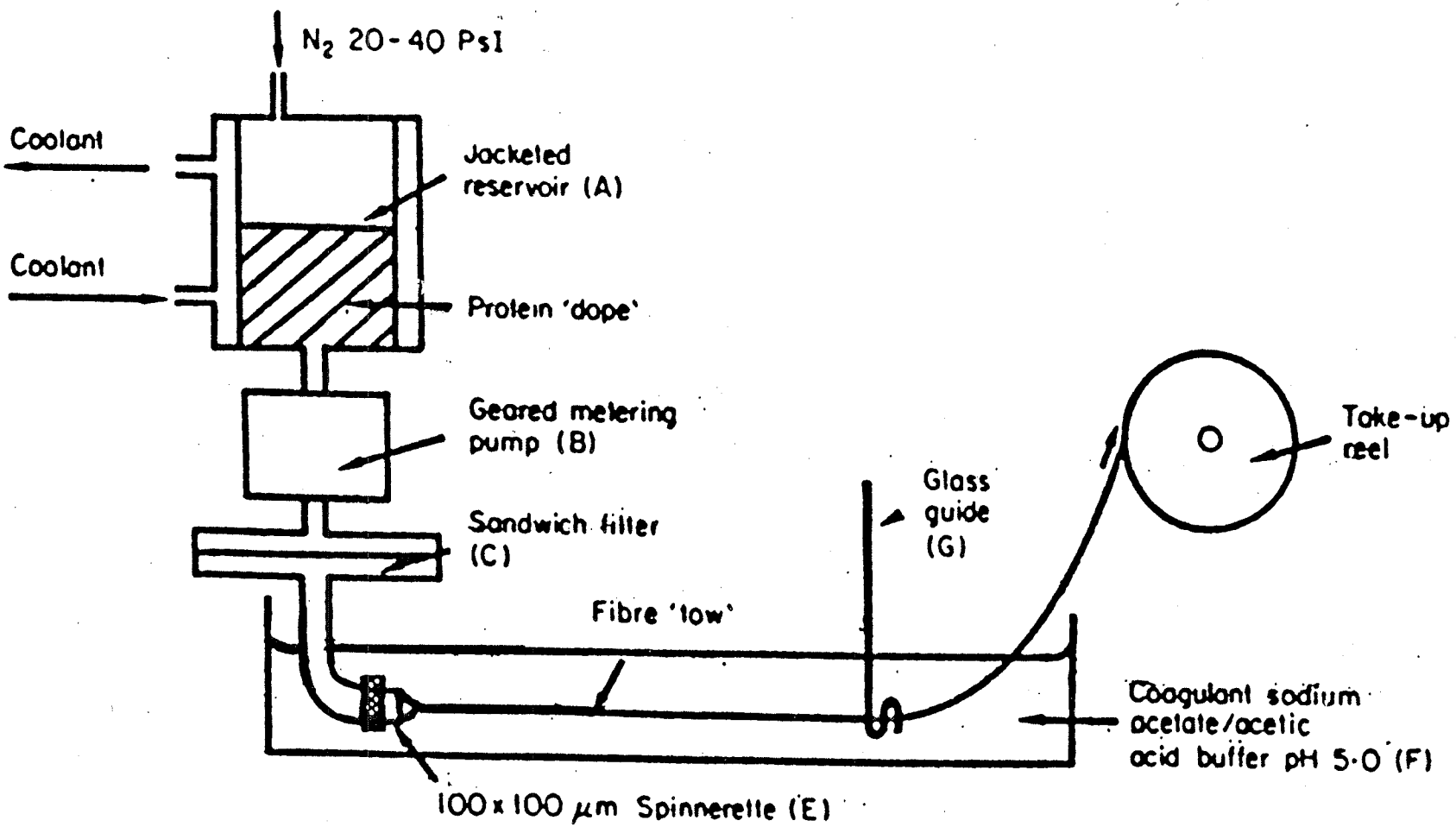
2.1.1. Spinning Procedure

As early as 1857, a British patent was granted for the spinning of protein fibres but not until 1935 did the

process for producing the protein fibres become commercialized (Balmaceda and Rha, 1974). These synthetic fibres were formed from animal proteins such as casein and keratin and were used mainly in the textile industry (Hartman, 1977). Since synthetic textile fibres could be formed from animal proteins, it soon followed that fibres could also be formed from such vegetable proteins as soy, corn and peanuts. The first application of this technology to the food industry involved the production of edible synthetic meats from vegetable proteins by Boyer in 1954 (Balmaceda and Rha, 1974; Hartman, 1977; Martin 1975). As a result, this process was later referred to as the "Boyer Process". At the present time, the Boyer process is utilized in producing edible protein fibres from both animal and plant sources (Balmaceda and Rha, 1974; Hartman, 1977).

The Boyer process essentially involved preparing a protein extract and pumping the extract under pressure through a spinnerette to form filaments of the desired texture (Balmaceda and Rha, 1974; Mackie and Thomson, 1982)(Fig. 1). The spinnerette, which is sometimes referred to as a porous membrane, contains 100 to 200 holes usually of 100μm diameter (Mackie, 1982; Martin, 1975). The spinnerette is submerged in a coagulating bath such that when the "extract" passes through the spinnerette, contact with the coagulating bath causes instant coagulation. As a result, the liquid jet of extract is

Figure 1. A typical spinning apparatus (Mackie and Thomson, 1982)


N_2 20-40 PsI
Coolant
Coolant
Jacketed reservoir (A)
Protein 'dope'
Geared metering pump (B)
Sandwich filter (C)
Glass guide (G)
Take-up reel
Fibre 'tow'
Coagulant sodium acetate/acetic acid buffer pH 5·0 (F)
100 x 100 μm Spinnerette (E)

transformed into a solid fibre and is then pulled or "towed" away from the spinnerette by a take-up reel (Castaigne et al., 1983; Mackie and Thomson, 1982). Two critical steps necessary for the proper operation of the spinning apparatus include:

i. Preparation of a protein extract
ii. Preparation of a coagulating medium

2.1.2. Preparation of the Protein "Extract"

Although the Boyer process initially involved producing protein extracts from various vegetable sources, several studies have been undertaken to produce protein extracts from the muscles of various fish species (Tanaka et al., 1983). Protein extracts from fish muscle have the advantages of being relatively inexpensive to produce (due to the low cost of the raw material) as well as displaying high nutritional values and superior textural qualities (ie. colloidal properties) (Lanier, 1986).

Fish protein is a composite of sarcoplasmic, connective tissue and structural proteins - however, only the structural protein fraction has any significance in preparing functional protein extracts due to its superior colloidal properties (Babbitt, 1986; Graham, 1977; Lanier, 1986; Spinelli et al., 1975). Spinelli et al. (1975) and Graham (1977) reported that the structural proteins generally comprise 70 to 80% of the total protein in fish muscle. Over 90% of the structural protein fraction is

comprised of actin and myosin (ie. myofibrillar proteins) while the remaining 10% contains mainly tropomyosin. In general, the myofibrillar protein fraction gives fish protein its characteristic textural properties as well as its gel-forming abilities (Lanier, 1986; Spinelli et al., 1975).

Spinelli et al., (1975) claimed that the remaining non-structural protein components, mainly the sarcoplasmic and connective tissue proteins, were of little technological significance. The sarcoplasmic proteins are low molecular weight, water-soluble proteins that do not contribute to the water-holding capacity of the structural protein fraction. Lanier (1986) stated that the sarcoplasmic protein fraction was composed of:

i. heme-containing compounds, which usually impart undesirable colours and which are known to catalyze lipid oxidation during storage

ii. enzyme systems, such as trimethyl amine oxidase, which lead to the formation of formaldehyde

iii. other proteins, which demonstrate poor functionality in terms of water-binding capacity and gelation ability

Combined with the connective tissue protein, which has minimal importance, the nonstructural protein fraction has no significant role in the gel-forming ability or functional properties of the fish protein as a whole.

Initial preparation of the protein extract first involved washing the deboned and minced fish flesh to remove excess fat, blood pigments and most of the undesirable proteins. These undesirable proteins are comprised of sarcoplasmic proteins (water soluble) and connective tissue proteins. The structural proteins, which display superior colloidal properties, do not possess as great a degree of water solubility and therefore remain after the washing steps (Graham, 1977; Lanier, 1986).

The addition of a saline alkaline buffer solution to the pre-washed minced fish flesh effectively disperses or solubilizes the structural or myofibrillar protein fraction (Castaigne et al., 1983; Graham, 1977; Lanier, 1986). It was found that dispersion or solubilization of the myofibrillar protein fraction occurred best in dilute alkaline saline solutions with an ionic strength in the range of 0.2 to 0.8 and a pH from 5.5 to 6.5 (Graham, 1977; Mackie and Hartman, 1982; Murray et al., 1981). Suzuki (1981) claimed that a minimum salt (NaCl) concentration of 2.0% (0.34 M) was required to disperse the myofibrillar protein fraction. Too high a salt concentration (ie. 1.0 M) caused salting-out or the inability of the myofibrillar proteins to become dispersed.

Graham (1977) noted that the dispersion of the fish protein was influenced not only by pH and ionic strength of the buffer, but by many other factors including nature of the fish protein (ie. fish species), physiological state of the fish and duration of extraction. It was found that

extracts from fish species with light coloured flesh (eg. whitefish) had superior functionality when compared to those species with dark-coloured meat (Lee, 1986). As well, the protein content of fish meat varied with the season. For example, Lee (1986) noted that fish caught during the feeding period produced a fish protein extract of superior functionality in comparison with fish caught during and after spawning. During the feeding period, fish muscle had the lowest pH as well as the highest total nitrogen level.

Castaigne et al. (1983) noted that the macromolecular protein structure of the extract became slightly denatured due to the action of the saline alkaline buffer solution. It was suggested that when subjected to a mild degree of denaturation, the protein molecules, which were originally in a tightly wound bundle, began to unwind into a linear orientation. This theory also claims that the unwinding effect results in improved textural characteristics of the formed fibre.

Spinning requires the use of purified protein isolates. In general, most proteinaceous isolates must have a minimum protein content of approximately 90%, dry basis (d.b.). to be spun using the Boyer process. It was found that when working specifically with fish protein, isolates required a minimum protein content of 70% in order to be spun directly (Hartman, 1978; Mackie and Thomson, 1982; Magnat and Bertrand, 1980). The minimum allowable

percentage of protein in fish extracts when compared to other animal or vegetable extracts can probably be attributed to the superior colloidal properties of fish protein. In general, fish protein isolates containing less than 70% protein (d.b.) produced fibres that were generally too soft and weak to handle. Increasing the protein concentration increased the ease and extent of cross-linking between exposed electrostatic, hydrogen, covalent and ionic bonding sites thereby promoting protein molecular interaction (Ziegler and Acton, 1984).

To produce extracts containing a sufficient protein content, a concentration step was required. The concentration step effectively removed much of the remaining and unwanted water soluble protein fraction (ie. sarcoplasmic fraction) as well as the excess water. However, overconcentrating the protein extract should be avoided. Over-concentration results in the reduction in the fluidity of the extract thereby reducing the level of pumpability through the spinning system, especially through the nozzles (Mackie and Thomson, 1982). When the extract maintained a sufficiently low viscosity, proper flow through the small nozzles of the spinnerette was attained. From previous work carried out by Askman et al., (1982) and Mackie and Thomson, (1982), it was discovered that these requirements were met when the protein content of the wet-based fish extract lay between 3 to 4% (w.b.). A protein content of approximately 3 to 4% corresponded to an approximate viscosity of 50,000 to 100,000 cps, which was

found to be acceptable for the spinning of fibres (Magnat and Bertrand, 1980).

2.1.3. Rheological Properties of the Protein Extract

In general, fish protein extracts are known to exhibit minimal thixotropic behaviour (ie. viscosity lessens with time) when exposed to shear force effects for extended periods of time (Magnat and Bertrand, 1980; Mackie and Thomson, 1982). This behaviour is evidenced by non-Newtonian pseudoplastic flow as characterized by a nonproportional relationship between shear rate and shear stress (Mackie and Thomson, 1982; Ismond et al., 1985). Several mathematical models, such as Casson's equation and the Power function, have been developed to relate shear rate to shear stress (Mackie and Thomson, 1982; Murray and Ismond, 1984). Mackie and Thomson (1982) also noted that due to the non-Newtonian nature of the fish protein extract, a nonlinear relationship existed between yield stress and protein concentration. It was discovered that the yield stress displayed an increasingly logarithmic dependance with increased protein concentration. This implies that a gradual increase in protein concentration may lead to an exponential increase in shear stress. This ultimately translates to eventual design constraints limiting the range of protein concentration over which the requirements for spinning and pumping can be met (Mackie and Thomas, 1982).

In addition, Magnat and Bertrand (1980) stated that

for protein in general, the maximum length of time the extract can be exposed to any form of shearing effects was dependant on such factors as temperature, type of protein, type of shearing equipment and the rate of shear. With respect to temperature, Mackie and Thomson (1982) found that fish protein extracts exhibit thixotropic behaviour when exposed to temperatures above 5°C. However, as the temperature was elevated above 5°C, the rate at which rheological change occurred also increased but not necessarily in proportion with temperature.

The rheological properties of fish extracts were also influenced by its shear history and by the length of time the fish had been stored in ice prior to protein extraction (Lee, 1986; Lee and Toledo, 1982). It was discovered that freshness was primarily time-dependant under given handling conditions. The fresher the fish, the better the functionality of the protein gel. The gel displayed the greatest functionality when the fish was processed within 1-2 days, provided it was stored in ice, or at near zero temperatures (Lee, 1986; Mackie and Thomson, 1982). According to Mackie and Thomson (1982), fish may be stored on ice for up to 9-12 days but a less viscous extract is produced. Although no reasons have been established, contributary factors are likely to include a variable degree of protein hydrolysis caused partially by contamination with visceral proteolytic enzymes during on-ship gutting procedures. Lee and Toledo (1976) claimed

that fish muscle contains highly unsaturated triglycerides, lipids, phospholipids, volatile carbonyls and considerable levels of nitrogenous components (urea) which are involved in the hydrolysis of the protein fraction thereby reducing the functional properties of the material.

2.1.4. Fibre Formation and Coagulation

Molecular Structure:

The basic structural requirements for a polymer to be fibre forming, as outlined by Hartman (1978) and Mackie and Thomson (1982), are:

i. High molecular weight (greater than 10,000)
ii. Long linear chain length
iii. High degree of linear symmetry
iv. High degree of polarity

These structural requirements are necessary in most cases for the development of proper protein orientation and gelation among the molecular chains. If the chain length is maintained, nearly all the structural proteins, when properly prepared, would be fibre forming under carefully established and selective chemical conditions (Hartman, 1977; Mackie and Thomson, 1982). It should be pointed out that depending upon the type of protein to be isolated (ie. animal, vegetable or fish) it may be required to adjust the pH of the extract to obtain optimum coagulating conditions. Typically, fish protein extracts undergoing coagulation in an acid-salt bath should have a pH within the range of 10 to 13 (Askman et al., 1981).

Once properly formed, the protein extract was loaded

into the spinnerette where under sufficient pressure, the extract was forced through the nozzle of the spinnerette. A hypothesis developed by Castaigne et al.(1983) and Lundgren (1949) claimed that during flow through the nozzle, the chains of protein molecules began to unwind from a bundled state into a linear orientation. It was suggested that this linear effect was necessary for the proper formation of solid fibres. Castaigne et al. (1983) claimed that the linear orientation of the protein chains began with the slight denaturing effect caused by the saline-alkaline buffer solution. At this stage, the protein chains, which were originally in a tightly wound bundle, began to unwind into a linear orientation. The mild degree of linear orientation, initially induced by the buffer solution, was further induced during flow through the spinnerette. After the extract passed through the spinnerette, the protein chains became aligned in a relatively linear pattern favouring molecular interaction and ultimately leading to fibre coagulation.

According to Ziabicki (1967), the solidification of the fibre by coagulation must be rapid in order to prevent the disorganization of the structure that was formed by its flow through the spinnerette. Based upon these theories, it appears that the process of fibre coagulation holds considerable importance in the overall textural qualities of the formed fibres as well as their eventual application into a food product.

Acid-Salt Coagulation

Acid-salt coagulation starts as the spun protein extract emerges from the spinnerette and comes into contact with the coagulating bath. Traditionally, the coagulating bath consists of a specific acid-salt solution. Either sodium chloride, calcium chloride or sodium acetate at varying concentrations ranging from 0.5 to 20% have been used as the salting agents (Ackman et al., 1982; Kuramoto and Thulin, 1970; Magnat and Bertrand, 1980; Tanaka et al. 1983). The presence of salt in such high concentrations was essential for allowing optimal coagulation by reduction of the pH of the isoelectric point of the fish proteins from its natural value of approximately 5.0 to a value of 3.0 to 3.5. Either acetic, phosphoric or hydrochloric acid are generally used in concentrations of 0.5 to 10% by weight as the acidifying agents (Kuramoto and Thulin, 1970; Magnat and Bertrand, 1980). A successful acid-salt coagulation medium specifically developed for fish protein (with a pH of 10 to 13) displayed an ionic strength and pH ranging from 2.0 to 2.2 and 0.7 to 0.9, respectively (Askman et al., 1981). Fish fibres having a pH within the range of 4.0 to 4.2 were thus obtained.

Coagulation was affected by many independent parameters including composition of the coagulating bath, (ie. pH and concentration), composition of the extract, coagulating properties of the protein extract, and geometry of the spinnerette (Balmaceda and Rha, 1974; Castaigne et al., 1983). The temperature at which the

acid-salt coagulation step is carried out may vary within wide limits, generally from 5°C to 80°C, but for ease and simplicity this step has usually been carried out in the region of ambient temperature or 20°C to 25°C (Kuramoto and Thulin, 1970; Magnat and Bertrand, 1980).

The critical step in the process of the acid-salt coagulation step was the diffusion of the cations (acid-salt coagulating agents) from the coagulating medium into the fibre and the diffusion of the water molecules from the fibre into the coagulating medium (Castaigne et al., 1983; Hartman, 1978). The cations act as electrolytes promoting the electrostatic bonding of protein molecules into a gel-like network structure (Castaigne et al., 1983). A good spinning design requires sufficient residence time for coagulation; this was found to depend on the diffusion coefficients of the acid-salt electrolytes through the protein fibre. Since the degree of diffusion of the electrolytes through the fibre strongly influenced the rate of fibre coagulation, this effect was believed to dictate the textural qualities imparted to the finished product (Castaigne et al., 1983; Hartman, 1978).

During coagulation, the fibre is characterized by two distinctive zones - the outer, or coagulated zone and the inner, or the noncoagulated zone, which are separated by a front that progresses towards the interior of the fibre (Castaigne et al., 1983; Hartman, 1978). Mathematical models have been developed by Castaigne et al. (1976) which

predict the coagulation time for different fibre diameters in different coagulating media. The models use Fick's Law and the diffusion coefficients of the electrolytes through the protein fibre. The ability to mathematically predict the rate of coagulation would aid in the determination of a minimum residence time for fibre coagulation.

Thermal Coagulation:

Two different methods involving heat have been used by Murray et al. (1981) and Askman et al. (1982). to coagulate fish and vegetable protein fibres, respectively. The first method by Murray et al. (1981) used ordinary tap water with a pH of 5.5 to 7.5 and a temperature of 90 to 100°C to coagulate various plant protein fibres. The fibres, which measured 0.4 mm in diameter, were immersed in the hot water bath for 0.5 to 1.0 minutes. It was claimed that a minimum temperature of 90°C was required since water temperatures below 90°C did not satisfactorily coagulate the plant protein fibres. Previous work by Wolf (1971) involved extrusion of soy protein into a 90°C water bath where it was held for 1 minute. The coagulated fibres displayed exceptional textural qualities which compared favourably with "meat" fibres.

The second method, as developed by Askman et al. (1982) involved an initial coagulation step in an acid-salt medium (required for protein fibres very susceptible to water) after which the fibres were immersed into a heated water bath. The fibres were then placed into a heated water bath (50 - 75°C) for 10 to 15 minutes. This step acted as a

secondary coagulating step in improving the textural qualities of the formed fibres as well as reducing their salt content (Askman et al., 1982). In these studies, it is important to recognize the sequence of events; the formed fibres must be initially coagulated (ie. in an acid-salt medium) before they can be immersed into a heated water medium. Once the acid-salt medium effectively coagulates the exterior of the formed fibre to a sufficient degree, subsequent immersion into water does not result in excessive solubilization or softening of the fibres. It appeared that a water medium heated to 50-75 °C was incapable of solely coagulating the formed fibres before they became excessively solubilized. The fibres were then neutralized to a pH of 6.0 - 6.8 by brief (3 to 10 seconds) immersions into an ambient temperature buffer solution of sodium bicarbonate. Askman et al. (1982) claimed that neutralization to pH 6.0 - 6.8 produced softer fibres which displayed a higher degree of rehydration when compared to fibres which were not neutralized.

2.1.5. Fibre Stretching

As part of the Boyer process, the fibres are stretched while simultaneously undergoing coagulation in the coagulating medium (Hartman, 1977; Balmaceda and Rha, 1974). The stretching action is carried out by a tension take-away reel which draws away the fibres at a speed greater than the rate of expulsion from the spinnerette. The stretching step acts to preserve the macromolecular

orientation of the formed fibre developed by the flow of the protein extract through the spinerrette (Castaigne et al., 1980; Hartman, 1978). By increasing the orientation rate of the macromolecules, it was found that the stretching action imparted a greater textural strength as well as increased tensile strength or elasticity (Castaigne et al., 1980; Hartman, 1978). During the stretching step, fibre elongation occurred within a short distance after the spinnerette; as a result, this region was considered the critical area which controlled the degree of macromolecular orientation within the fibre.

After coagulation was carried out in an acid-salt medium, it became important to wash the fibres of residual inorganic salts. This step was necessary because it was previously discovered that unwashed fibres acquired a "tough" texture as well as a salty or acetic taste (Castaigne et al., 1980; Magnat and Bertrand, 1980). After washing, the fibres obtained were white in colour with a gel-like property (Mackie and Thomson, 1982).

2.2. TEXTURIZATION THROUGH EXTRUSION

Until 1935, most extruders had been used and modelled from work conducted in the area of plastics extrusion (Harman and Harper, 1974; Rossen and Miller, 1973). The single-screw extruder had first been applied to food processing in 1935 for the continuous extrusion of pasta products. In 1946, the use of extruders in food processing developed rapidly with the introduction of simple corn

snacks produced by the Adams Company (Guy, 1985).

In general, extruders have the principle functions of mixing, shearing, pumping and forming food materials (Rossen and Miller, 1973; Stanley, 1986). Inherent in the extrusion process is a combination of one or more of these functions.

Screw extruders are classified into two categories - cold extruders and hot extruders. According to Williams et al., (1977) cold extruders are designed to extrude a material without the generation of heat. For this reason, cold extruders usually have slow turning screws designed for low shear rates and are used for restructuring heat sensitive products. Hot extruders are designed to generate heat by shear friction developed by a rapidly rotating screw working on the material to be extruded. Hot extruders have the added functions of gelatinization/cooking, sterilization and puffing or drying (Harman and Harper, 1973).

2.2.1. Extrusion Process

According to Rossen and Miller (1973), a typical single screw extruder operation begins with food material being fed into a hopper; this subsequently passes through the feed throat and into the channel of the screw. The screw rotates in the barrel which contains a hardened liner. The motor drives the screw through a gear reducer and the backward thrust of the screw is absorbed by the thrust bearing. As the food material is conveyed along the

screw channel, it is subjected simultaneously to mixing, heating and shearing; as it nears the discharge end (ie. near the die), it is transformed gradually into a thermoelastic, viscoelastic material (Fig. 2).

2.2.2. Raw Material

Since food properties are far more complex than those of plastics, the theories regarding plastic flow cannot be directly used to define the extrusion of food materials (Rossen and Miller, 1973). For example, food materials do not simply melt as do plastics. As well, irreversible time dependant changes further complicate the rheology of foods during extrusion.

Rossen and Miller (1977) claimed that the three main limitations affecting the design of food extruders are - the rheology of the food material, the geometry of the screw and the operating variables. To overcome these limitations, food extruding operations are simplified by the following assumptions:

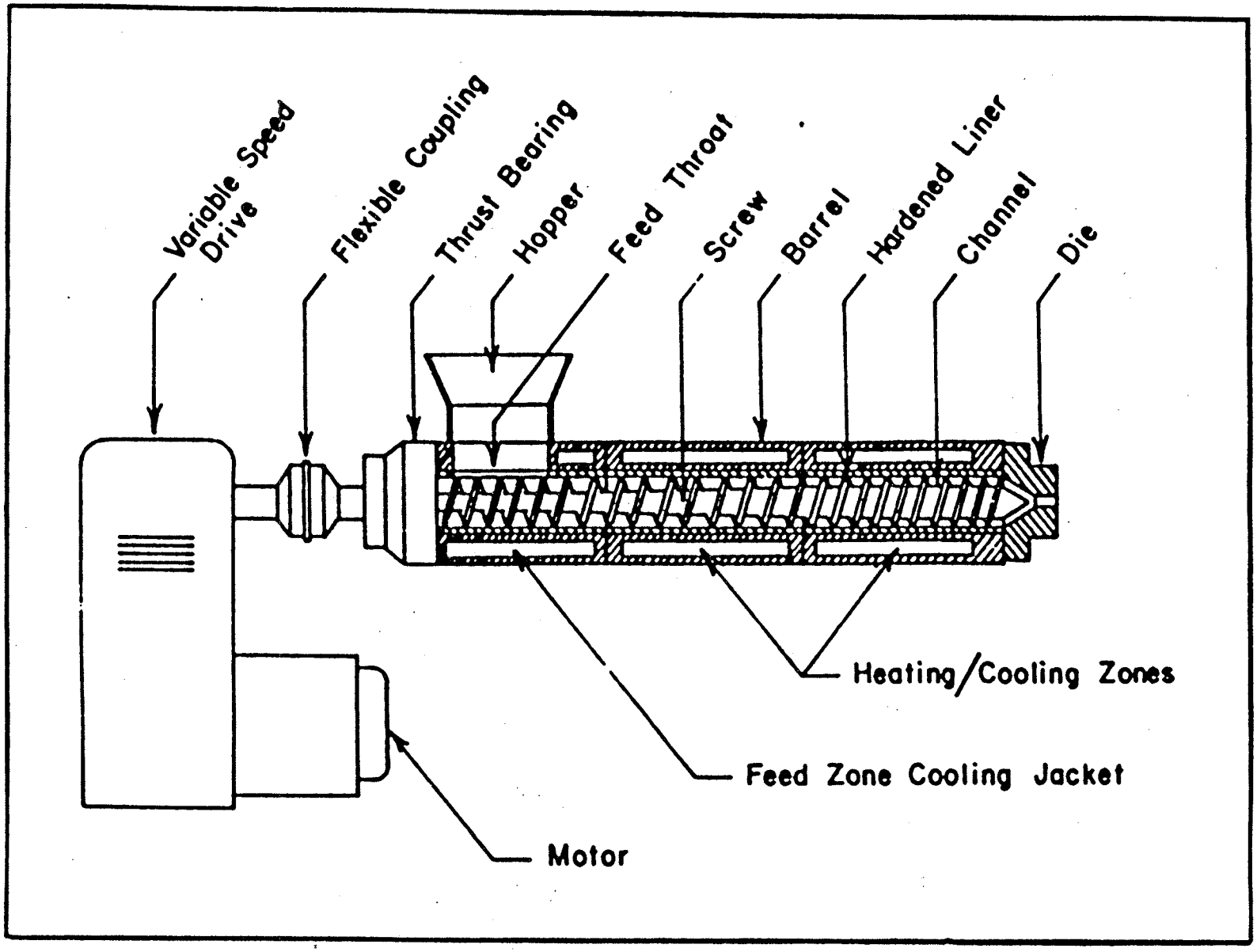
i. the food product is Newtonian in nature

ii. the screw geometry is simple and constant throughout its length

iii. the barrel fits tightly over the screw thereby eliminating backflow losses

These assumptions, as stated by Rossen and Miller (1973) and Clark (1978), helped in modelling the action of an ideal single-screw extruder used to qualitatively predict the pattern of material flow.

Figure 2. A typical single screw extruder (Rossen and Miller, 1973)


Variable Speed Drive
Flexible Coupling
Thrust Bearing
Hopper
Feed Throat
Screw
Barrel
Hardened Liner
Channel
Die
Heating/Cooling Zones
Feed Zone Cooling Jacket
Motor

Due to the importance of the flow patterns, the most critical physical properties of a proteinaceous material undergoing extrusion is the apparent viscosity (Clark, 1978; Williams et al., 1977). The apparent viscosity of most food materials was found to be highly non-ideal and was affected by composition, shear history of the material, time, temperature and shear rate. One unique feature of food extrusion in contrast to most other thermoplastic extrusions is the fact that the apparent viscosity of food materials increases with cooking and reaction time while most polymers experience a reduction in viscosity as the temperature rises. As a result, when referring to the composition of food materials, it appears that the moisture content has a very strong effect on the apparent viscosity and should be considered as a major processing variable (Clark, 1978). In addition, moisture levels also influence the relative ease of feeding food material into the extruder (ie. food materials with high moisture contents are relatively easy to feed into an extruder).

2.2.3. Textural Changes Imparted Through Extrusion

The screw extruder is unique in its ability to texturize typically viscous, proteinaceous material as it travels through the extruder. The changes in the starting material during flow through the extruder barrel can best be understood by picturing the flow paths of the material. According to Clark (1978) and Harper (1986), if the extruder screw were to be unwound, it would present a long

narrow rectangular channel that behaved as if it had a flat plate moving across it at an angle. Since the effective movement of the channel relative to the plate (representing the barrel surface) was at an angle, the velocity of the material in the channel could be resolved into two components - one linearly down the channel and one perpendicular to it or across the channel. Since the net flow across the walls of the channel must be zero, a cross channel velocity component results which creates a circular flow across the channel; flow along the top of the channel would be in one direction while flow along the bottom of the channel would be in the opposite direction. This flow behaviour results in every particle following a helical path thereby contributing to a physical molecular uniformity which is helical in structure. The food particles following the helical pathway orient themselves into a fibrous or laminar structure while in the extruder barrel. Laminar flow dictates that adjacent layers of the food particles move relative to one another in parallel paths without the occurence of radial intermixing.

Clark (1978) and Harper (1986) claimed that during extrusion, the proteinaceous starting material oriented itself into a linear, fibrous structure simply by following the flow in the extruder barrel. Thus, the extrusion process distinguishes itself from other forms of texturization (ie. spinning) in that texturization occurs before flow through the die.

In preparing texturized analogue materials, screw

extruders require that the starting material being fed into the hopper have a relatively low moisture content (or alternatively, a high viscosity) with a texture similar to that of a thick paste or dough (Clark, 1978). For proteinaceous materials, optimal moisture levels have been found to vary with the quality of the protein ingredients, but generally range between 13-45% (d.b.) (Harper, 1986). Materials having higher moisture contents cannot effectively be restructured due to the inability of the product to undergo the helical shearing effect as evidenced with higher viscosity materials. For example, within the food industry, soy pastes having moisture contents of 10 to 20% (d.b.) have been successfully texturized into a fibrous structure and used in simulated meat products and extenders (Clark, 1978).

A slightly modified use of the extruder has been applied to the extrusion of fish paste or surimi. According to Lee (1986), a single screw isothermal extruder was first used by the Japanese to extrude surimi through a small rectangular die, producing a rectangular sheet. An alternate procedure involves extruding a sheet of the surimi material which is then partially heat set (80 to 90°C) and scored into strips or "fibres". This particular process is in contrast to conventional extruder texturization processes whereby the starting material is texturized while in the extruder barrel prior to its exit through the die.

2.3. APPLICATION

Once a suitable texturization method is implemented for a particular raw material, a variety of simulated food products may be produced. As well, food extenders are used to restructure and increase the textural qualities of various seafood, meat and poultry products. The end result is a low-cost, uniform, completely edible food product that resembles fresh intact muscle in textural, flavour and colour properties (Mandigo, 1986).

In particular, when simulating various seafood based analogues, the closer the simulation desired, the greater the sophistication of the processing techniques required (Lee, 1986). Up until 1985, Japanese innovators had developed more than 60 surimi-incorporated products ranging from the popular simulated shellfishes to fish sausages, surimi noodles, beef jerky and bread (Roche, 1985). One of the most commercially successful "fiberized" seafood analogues were "crab sticks" and "scallops" produced using a modified extrusion process, originally developed in Japan (Lee, 1984; Roche, 1985).

2.3.1. Fabricated Crab-Flavoured Sticks and Scallops:

The fabrication process as described by Lee (1984) begins with fish paste being extruded through a rectangular nozzle having a narrow opening (1-3 mm gap) into a thin sheet. The sheet is then partially heat set and scored into desired widths. Strip width is determined

by the type of finished product desired. Narrow strips were preferred for the fibrous crab-leg products whereas wider strips became more suitable for the simulated scallops or other shellfish in the form of seaflakes or chunks. The scored sheets are then folded or rolled into a rope by a simple narrowing device called a rope former. The rope was then coloured, wrapped and cut into desired lengths. Lee (1984) also noted that since 1980, many improvements have been made in these fabricated products and as a result, consumer acceptance of this line of product has increased.

A desired fibrous texture could also be achieved by a combination of fiberization and textural modification through the use of ingredients, mainly starch and egg albumen. According to Lee (1986), up to a level of 10% (weight basis) starch tended to increase cohesiveness and rigidity of the product. Egg albumen caused the product to become significantly firmer, more cohesive and less elastic thereby making the product more brittle and less elastic after cooking. From a sensory aspect, starch tended to maintain the rubberiness of surimi gel by reinforcing the composite matrix of the protein gel network. Egg albumen, on the other hand, reduced the rubberiness by interfering with the myofibrillar cross-linking process and thus disrupting the composite gel matrix. Thus, it appears that starch and egg albumen counteract each other. However, to impart a not excessively rubber but yet fibrous texture, it was necessary to include both ingredients into the formula.

Furthermore, Lee (1986) noted that starch, and to a lesser degree egg albumen, increased the freeze-thaw stability of surimi by reducing the effects of drip loss which caused a spongy texture.

Several companies using this technique to produce a variety of seafood analogues include Kibun International of Japan, JAC Creative Foods Inc. of California and Terra Nova Fishery Co. Ltd. of Newfoundland (Apold, 1984; Roche, 1985). Terra Nova Fishery Co. Ltd. began production of crab sticks primarily from Atlantic cod; these are marketed by Kraft Canada bearing the name "Harbour House Kamaboko" (Roche, 1985). Although this process has proven to be commercially successful, Lee (1984) noted that there is scope for improvement, particularly in the texturization techniques. Sensory panelists cited major textural differences between real crab leg and simulated crabsticks (JANA Brand) produced using the above mentioned extrusion process (Ismond et al., 1985). For example, there were significant differences between the two products with respect to the products' perceived fibre diameter, chewability, hardness and juiciness.

2.3.2. Fabricated Shrimp

It was reported that considerable success had been attained by Lanier at North Carolina State University in simulating seafood items (Anonymous, 1980). A high-speed, reciprocating nozzle, low-temperature extruder was used to fabricate surimi-based products shaped in the form of

shrimp. Two shrimp simulations were produced; one composed of half shrimp and half surimi while the second was composed of all shrimp. The simulated shrimp was compared to whole breaded shrimp and two commercially fabricated shrimp products. Based on firmness, springiness and cohesiveness, the evaluation by a trained panel showed that the extruded half shrimp, half surimi product scored highest in simulating whole breaded shrimp. The high rating of this product was attributed to the increase in firmness and springiness attributable to surimi. The surimi-containing product was readily formed into uniform shapes in the extruder without the need of an additive binder matrix. The all shrimp fabricated product required a binder matrix (bread crumbs and hydrocolloid) to enable proper shaping. This product lacked the firm, springy texture characteristic of the whole breaded shrimp.

A high-pressure, high-temperature injection molding process has been developed to fabricate jumbo size shrimp from tiny shrimp. According to Ellis (1983), the process began with peeled shrimp which were "medium" ground in a conventional meat grinder. The ground shrimp were injected under 12,000 psi pressure into a series of mold assembly cavities. The injected shrimp were flash heated for 6 seconds at 80°C thereby building up internal pressures and causing the protein fibres to knit together uniformly. No gums, extenders or any other binding agents were used. Ellis (1983) claimed that the finished product had the dense, firm texture characteristic of the natural product.

This process is being successfully carried out by Bee Gee Shrimp Co., Inc. in Lakeland, Florida. This particular plant has the capacity of producing 250 kg/h of molded "Shrimp Perfects" at approximately half the cost of whole natural jumbo shrimp (Ellis, 1983).

More recently, Andres (1984) reported that studies carried out at ABC Research (Gainesville, Florida) had shown that certain methyl-cellulose food gums, primarily Methocel A4M Premium, could be beneficial in the production of low-temperature extruded/formed seafood products. Of particular interest was the emulsion made up of approximately 45% shrimp and 31% surimi (turbot species). The gum functioned best if incorporated into a matrix system containing bread crumbs and potato starch. Addition of the gum matrix system to the seafood emulsion allowed the use of a low-temperature extruding/forming process. The study compares this process favourably to other extruding/forming processes where heat is required to partially heat-set the product in order for the product to retain its original form.

2.3.3. Fabricated Lobster Sticks

Work carried out by C.M. Lee at the University of Rhode Island successfully combined salvaged lobster meat with surimi (Red hake species) to produce "Lobster Sticks" (Nolan, 1983). Lee found that an extrusion process, similar to that used to produce crab-flavoured sticks, resulted in a product with a texture similar to that of

real lobster.

2.4. SUMMARY OF LITERATURE

After studying the operating parmaters behind the spinning apparatus and the screw extruder, a series of fundamental operating parameters were selected based upon their potential application toward the eventual design of a fibre forming apparatus. It was hoped that the chosen parameters would aid as suggested guidelines during the design and fabrication of the proposed apparatus.

1. The fish protein extract should exhibit a viscosity between the range of approximately 50,000 cps (3-4% protein concentration, w.b.) to a maximum of 150,000 cps.
2. An extract with too high a viscosity results in reduced pumpability. Alternatively, an extract with too low a viscosity, corresponding to a low protein content, produces unacceptably weak fibres.
3. During flow through the spinnerette, the protein molecules of the extract theoretically align themselves into a linear pattern which ultimately promotes fibre coagulation. The degree of linear orientation was physically dependant upon the length and diameter of the spinnerette's nozzles. As a result, it was required that the optimum length and diameter of the nozzles be

determined.

4. The spinning process may include several different coagulating media such as an acid-salt medium, thermal medium (ie. heated water) or a combined acid-salt/thermal medium. However, from research carried out at the Department of Food Science (U of M), it was discovered that acidified ethanol provided an effective means to coagulate fish protein fibres (Ismond et al., 1984). A thermal medium could also be used in conjunction with this coagulation but like the acid-salt coagulation step, it must be preceeded by a chemical coagulation step to prevent premature solubilization of the fibre when immersed into the water medium. In one respect, using ethanol as a coagulating medium offered a definite advantage in that the fibres did not contain a salt residue, which was sometimes difficult to remove after coagulation in the acid-salt medium. Although coagulation in ethanol would also leave a residue, it would likely be easier to remove than the salt.
5. The coagulation process may occur at room temperature.
6. The viscosity of the fish protein extract is

expected to decrease when exposed to either extended periods of shearing forces or temperatures above 5°C. When stored at 0 to 5°C without exposure to any shearing effects, the viscosity of the extract increases.

7. If the extract had a sufficiently high viscosity, similar to that of a paste, it could theoretically be texturized using a screw extruder. The extruder should have the capability of simultaneously coagulating (thermally) the starting material as well as carrying out the required texturization process.

3. MATERIALS AND METHODS

3.1. EXPERIMENTAL MATERIAL

3.1.1. Preparation of Raw Minced Fish

Freshwater whitefish (Coregonus clupeaformis) was used as the base material for obtaining the minced fish. The fish was commercial cutter-grade quality supplied by the Manitoba Freshwater Fish Marketing Corporation.

Whole, dressed, deheaded fish were scraped and washed to remove most of the remaining blood and viscera. The fish were split into skin-on fillets with the backbone remaining on one fillet. The fillets were deboned using a Baader Flesh Separator equipped with a 5 mm diameter perforated drum. The minced flesh was washed with 4 times as much water as flesh (w/w) for 4 wash cycles. During washing, the flesh was mildly agitated for 2 minutes followed by a 2 minute settling period. As much water as possible was decanted from the minced fish after the settling period.

After washing, the minced flesh was packed into fine mesh polyester bags. The bags were hung for appoximately 1 hour to allow most of the excess water to drain. After draining, the Baader Flesh Separator, equipped with a 3 mm diameter perforated drum, was used to remove most of the undesireable material still present (ie. skin) in the flesh.

Food grade sodium chloride was added to the minced flesh (1% w/w) and mixed for 15 minutes using a Hobart LP-800 mixer. The blended minced fish was packed into 25 mm thick sheets (500x 300 mm) and quick-frozen in a double plate Dole Freeze-Cel contact freezer. After 3 hours, the sheets were removed, properly packaged and stored at -40°C until required.

3.1.2. Preparation of the Protein Extract

Homogenization

While frozen, the sheets of minced whitefish were shaved into thin (2-4 mm thick) strips using a sharp filleting knife. The shaved strips were added to chilled (0-5°C) food-grade, saline-phosphate buffer solution (0.5 M sodium chloride and 0.04 M sodium phosphate monobasic, adjusted to pH 7.2 with 1.0 M sodium hydroxide). Approximately 70-100 g of shaved fish were added to 400 ml of buffer solution. The fish was homogenized in the buffer using a Silverson, Standard Lab mixer equipped with an emulsor screen. Homogenization was carried out using three 2 minute cycles interspersed with 2 minute rest periods to allow heat dissipation. All steps of the procedure were carried out in a 4°C cold room.

Initial Centrifugation

The resulting homogenate was centrifuged using a Sorval Refrigerated Centrifuge, Model RC-5B, at 15000 x g for 20 minutes. The temperature was maintained at 2-5°C. The supernatant was decanted and diluted with an equal volume of chilled (0-5°C) distilled water. The suspension

was stirred for 5 min after which time it was further diluted with enough chilled, distilled water to double its volume. The suspension was allowed to stir for an additional 5 min.

Filtration

To remove any heterogeneous particulate matter still in the suspension, two C.E. Tyler Canada Standard Sieves (425 μm and 300μm) were used in series with each other. These effectively removed most of the particulate matter larger than 300μm from the suspension.

Final Centrifugation

The suspension was subjected to a 2-stage centrifugation process. In the first step, the suspension was centrifuged at 13000 x g for 30 minutes using a pilot plant model International Refrigerated Centrifuge in order to precipitate most of the protein from the water phase. The precipitated protein was then collected and recentrifuged for 10 minutes at 15000 x g in a Sorval Refrigerated Centrifuge, Model RC-5B. The resulting pellet, representing the fish protein extract, was recovered and adjusted to pH 8.2 using 0.1 M sodium hydroxide.

3.2. DESIGN AND FABRICATION OF A SMALL SCALE FIBRE FORMING APPARATUS

3.2.1 Determination of Initial Design Parameters

In order to design a prototype large scale fibre forming apparatus, it was necessary to carry out some

preliminary research. Therefore, a prototype Small Scale Fibre Forming Apparatus (SSFFA) was designed (Fig. 3). The apparatus consisted of an aluminum cylinder (35 mm in diameter and 30 mm in height), a piston-type plunger mounted onto an Instron Universal Testing Machine and an extruding die lying flat inside the cylinder. The plunger attached to the Instron exerted a force sufficient to extrude the protein extract through the die.

The parameters investigated included: optimum extruding pressure plus diameter and length of the die holes. The quality of the formed fibres was assessed visually while variations were made in the above mentioned parameters.

The protein extract was extruded into an aqueous coagulating bath of 95% ethanol with sufficient acetic acid to decrease the pH to 4.2. Two types of extruding dies were tested:

i) Straight Hole Die

ii) Hypodermic Needle Die

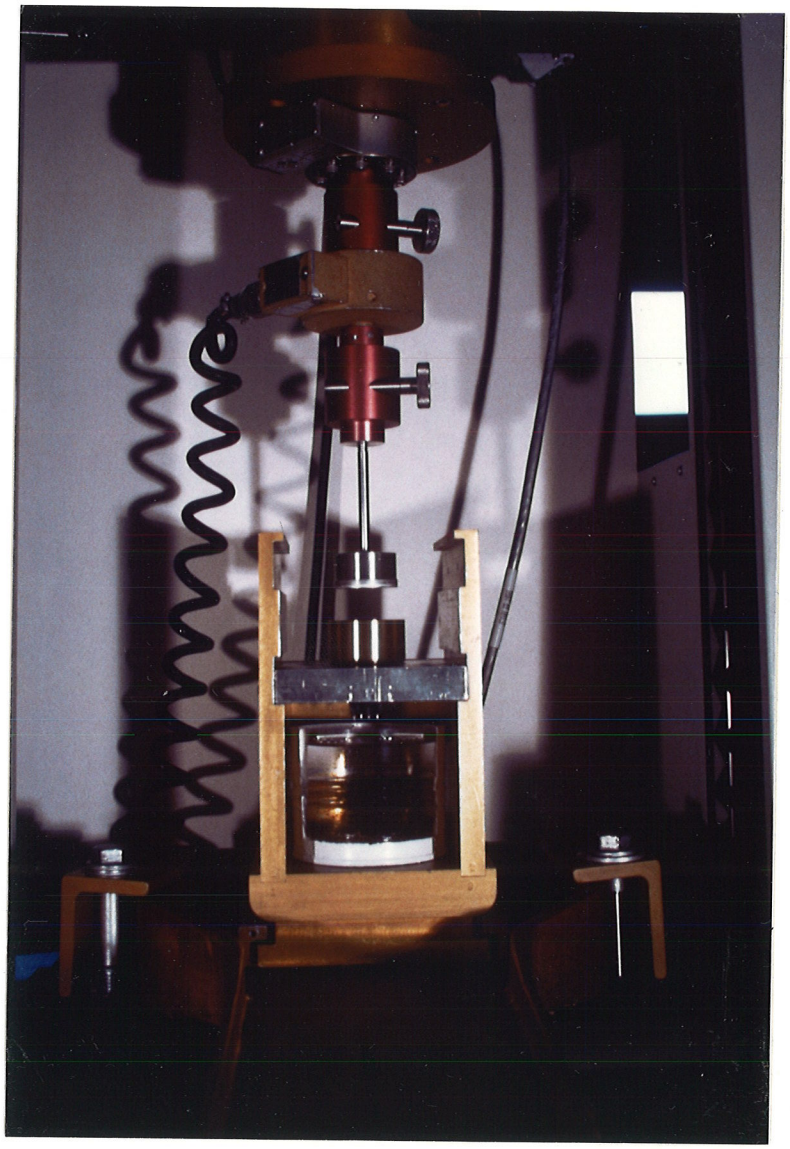
Straight Hole Die

A round aluminum plate with a diameter of 35 mm and thickness of 3 mm was used. Four straight holes of equal diameter were drilled into each plate. A total of 4 plates were made which contained hole diameters of 1.0, 0.8, 0.6, and 0.4 mm. Approximately 15-20 ml of protein extract (with pH adjusted to 8.2) were used for each run.

Hypodermic Needle Die

A circular aluminum plate with the same dimensions as

Figure 3. A small scale fibre forming apparatus (SSFFA) using a hypodermic needle die



that of the straight hole die was used. Four straight holes were drilled into the plate and disposable hypodermic needles were inserted into each hole. Needles of three different inside diameters were tested: 18 G (1.07 mm), 22 G (0.46 mm) and 26 G (0.31 mm). Fibre formation was attempted under a total of 12 extruding pressures, ranging from 100 kPa (15 psi) to 600 kPa (90 psi). For each of the 12 extruding pressures, the length of the 18 G and 26 G needles was sequentially reduced from 23 to 15 mm in 4 mm intervals. The length of the 22 G needles was reduced from 48 to 18 mm in 10 mm increments. Approximately 15-20 ml of protein extract (pH adjusted to 8.2) were used for each run.

3.3 TESTING OF A SCREW EXTRUDER AS A POTENTIAL FIBRE FORMING APPARATUS

Once preliminary testing of the SSFFA was completed, the results were applied towards the operation of a screw extruder as a fibre forming apparatus. A single screw, isothermal Defrancisci Spagetti Extruding Machine, model S-25, was tested as a potential fibre forming apparatus. A multiple nozzle extruding die was designed and fabricated to attach onto the discharge end of the screw extruder. The die consisted of a round brass plate (114 mm diameter) with a total of forty 22 G hypodermic needles inserted into it in a concentric pattern. The extruder was equipped with a variable speed pulley which could regulate the rotational speed of the screw thereby regulating the applied pressure.

Approximately 2 litres of fish protein extract was prepared for the trial attempt at fibre formation. This extract had the consistency of a paste with a viscosity of 140,000 cps, as measured by a Brookfield viscometer.

Attempts at forming fibres using the screw extruder proved unsuccessful due to the inability of the screw to carry the material through the barrel. As a result, it was decided that a new fibre forming apparatus was required.

3.4. LARGE SCALE FIBRE-FORMING APPARATUS

Based upon the results of the SSFFA as well as the unsuccessful attempt at forming fibres using the pasta extruder, a new fibre forming apparatus was designed. This apparatus was referred to as a Large Scale Fibre Forming Apparatus (LSFFA). It consisted of a Moyno Pump Assembly (MPA) and a Fibre Conveying Device (FCD).

3.4.1. Moyno Pump Assembly (MPA)

The Moyno pump is a moving cavity pump with a stainless steel rotor rotating in a rubber stator. These pumps are designed specifically to handle a wide range of materials from water to thick pastes, particulates and slurries. The Moyno Pump Assembly (MPA) was made up of a quick disassembly Moyno pump, frame type 1FF6, which was powered by a 1.5 kW (2 hp) variable drive DC motor. The extruding die, originally fabricated for the pasta extruder, was modified to enable it to be attached onto the discharge end of the MPA. The die still consisted of a circular brass plate with forty 22 G hypodermic needles

inserted into it in a concentric pattern.

3.4.2. Fibre Conveying Device (FCD)

A conveying device was designed that would carry away the formed fibres from the MPA and yet allow sufficient time for the fibres to coagulate properly. The device consisted of Flat Flex wire belt (150 mm wide) travelling submerged through two separate and enclosed liquid media. The belt passed first through a coagulating medium then emerged onto a drip cycle and reentered later into a water wash medium. The fibres remained constantly submerged while travelling through the two media. During the drip cycle, a device known as a fibre bundler was incorporated to bundle the fibres into a "rope". The fibre bundler consisted of a triangular-shaped, 6.4 mm (inside diameter) hollow tubing. A total of sixteen, 0.4 mm diameter holes were drilled into the tubing at various angles such that the angle at which the air streams were directed, collectively blew the fibres into a rope. The fibre bundler required approximately 138 kPa (20 psi) of pressure for proper operation. Once the fibres were bundled into a rope, they passed through the water wash cycle to remove excess acetified ethanol and were later collected for further use.

3.5. TESTING OF FIBRE QUALITY

Most testing was carried out on the fibres produced specifically by the LSFFA. Individual fibres tested for tensile strength were formed by a bench scale procedure using a hypodermic needle (22 G) and syringe with identical

coagulating conditions as those used for the LSFFA.

3.5.1. Fibre Tensile Strength

Individual fibres were tested for their tensile strength immediately after being formed (fibres were submerged in the coagulating medium for approximately 50 sec). Each fibre was wrapped between two vertically oriented hooks. The top hook was mounted onto a 10 N Engineering Specifics Association Load Cell, while the bottom hook was fixed in a base mounted chuck (Fig. 4). The effective length of the fibres, or the distance between the hooks, was 30 mm. Tensile strength, defined as the maximum tensile stress that the fibre was capable of sustaining before rupturing (Finney, 1972), was measured after exposure of the fibres to various coagulating conditions and design parameters.

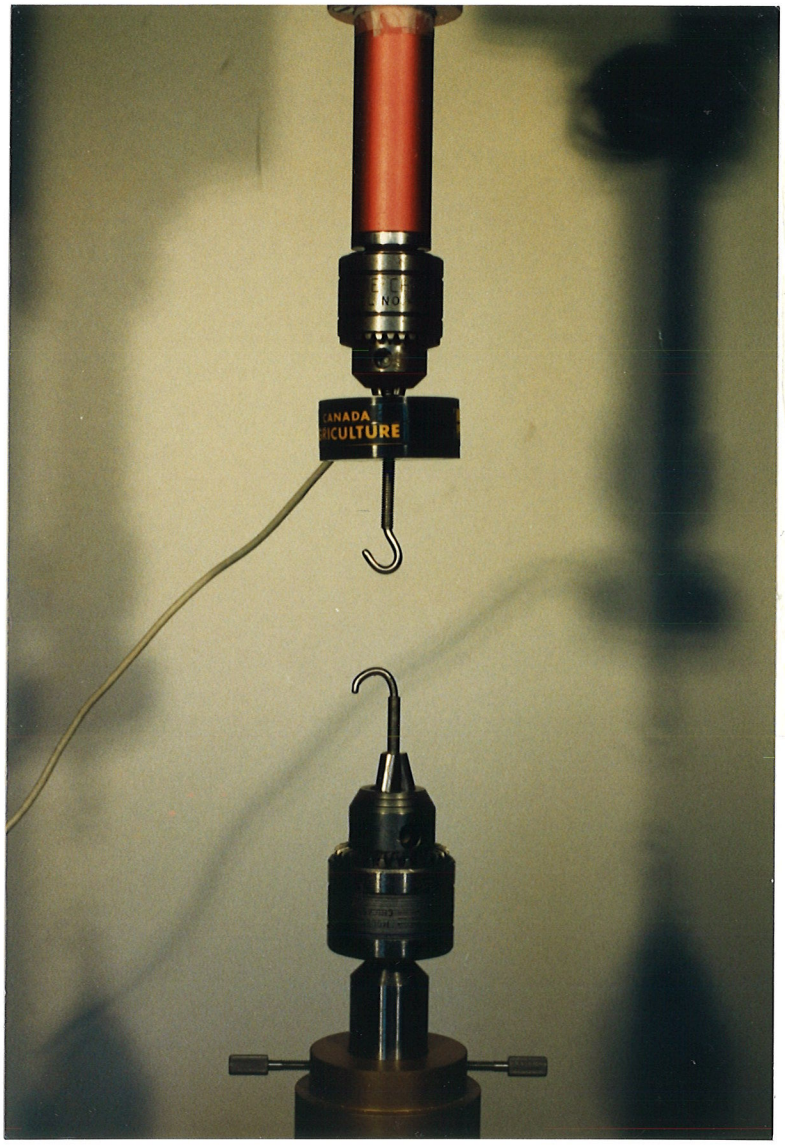
Effect of Not Washing Fibres After Coagulation

Fibres were coagulated in the conventional ethanol-acetic acid medium (pH 4.2) and tested for tensile strength immediately afterwards.

Effect of Washing Fibres After Coagulation

Fibres were coagulated in the conventional ethanol-acetic acid medium (pH 4.2). After the standard coagulating time of approximately 50 sec, the fibres were immersed in a water bath for approximately 5 sec. Fibres were tested for tensile strengths immediately after the washing procedure.

Figure 4. Load cell apparatus used to assess the tensile strength of individual protein fibres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Effect of Adjusting pH

The pH of the coagulating medium was adjusted from pH 4.2 to pH levels of 4.5, 5.0, 5.5, 6.0 6.5 and 7.0 using glacial acetic acid. As well, fibres were coagulated in an ethanol medium (pH 7.2) without any acetic acid.

Effect of Adjusting Ethyl Alcohol Levels

During this step, ethanol (pH 7.2), without acetic acid, was used as the coagulating medium. The original 95% ethanol was diluted to levels of 80, 70, 60 and 50% with distilled water.

Effect of Storage

Fibres were stored in the conventional ethanol-acetic acid medium (pH 4.2) for a range of times including 0, 1, 24 and 48 h.

3.5.2. Effect of Heating on Fibre Shear Strength and Cohesiveness

The fibre ropes formed by the LSFFA were packed in equal weights (16.5 g) into hollow, plastic cylinders. The cylinders were 25 mm in diameter and 60 mm in length. The fibres were packed such that they were aligned in a linear pattern along the length of the cylinder. Individual samples were thermally set using a CEM Corporation, model MDS 81, 600 W microwave system. Samples were subjected to microwave radiation for 2 minutes at power levels of 10, 20, 30 and 40%. Two Luxtron fibre optic probes inserted into each sample were used to measure interior temperatures at 30 second intervals during the two-minute heating process (Fig. 5).

Figure 5. Fibre bundle inserted with two fibre optic probes prior to microwave heating


CEM MDS 81
7 8 9
4 5 6
1 2 3
0
PROGRAM
ENTER
RESET
STOP
START
ON
OFF

After microwave exposure, the fibre ropes were removed from the plastic cylinders. The thermal effect caused by microwave exposure imparted a degree of cohesiveness between the fibre ropes as well as between individual fibres; as a result, the fibres remained as a cohesive bundle after removal from the plastic cylinders. The fibre bundles (retaining the characteristic round shape of the plastic cylinders) were then placed in plastic bags and allowed to cool. The diameters of the round fibre bundles were measured before further analysis was carried out.

Shear Strength

The fibre bundles were cut into 30 mm lengths and sheared using a Warner-Bratzler Shearing Device (Fig. 6). A 500 N Instron Corporation Load Cell-A mounted onto the Universal Testing Machine was used.

Bundle Cohesiveness

The fibre bundles were cut into 20 mm lengths. Cohesiveness of the fibre bundles was measured using a tensile test cell (Figs. 7, 8). The tensile test cell was a standard unit used in meat testing but modified by the addition of the two small pin plates seen in Figure 8. The tensile device consisted of four linearly aligned steel pins (1.0 mm diameter), spaced 5 mm apart and mounted into a plexiglass base. The pin plates were mounted onto a horizontal stretching device which consisted of one fixed and one sliding (assumed frictionless) platform. The sliding platform was attached to a 22.5 N Eaton Corporation

Figure 6. Single blade Warner-Bratzler apparatus used to assess the shear strength of fibre bundles

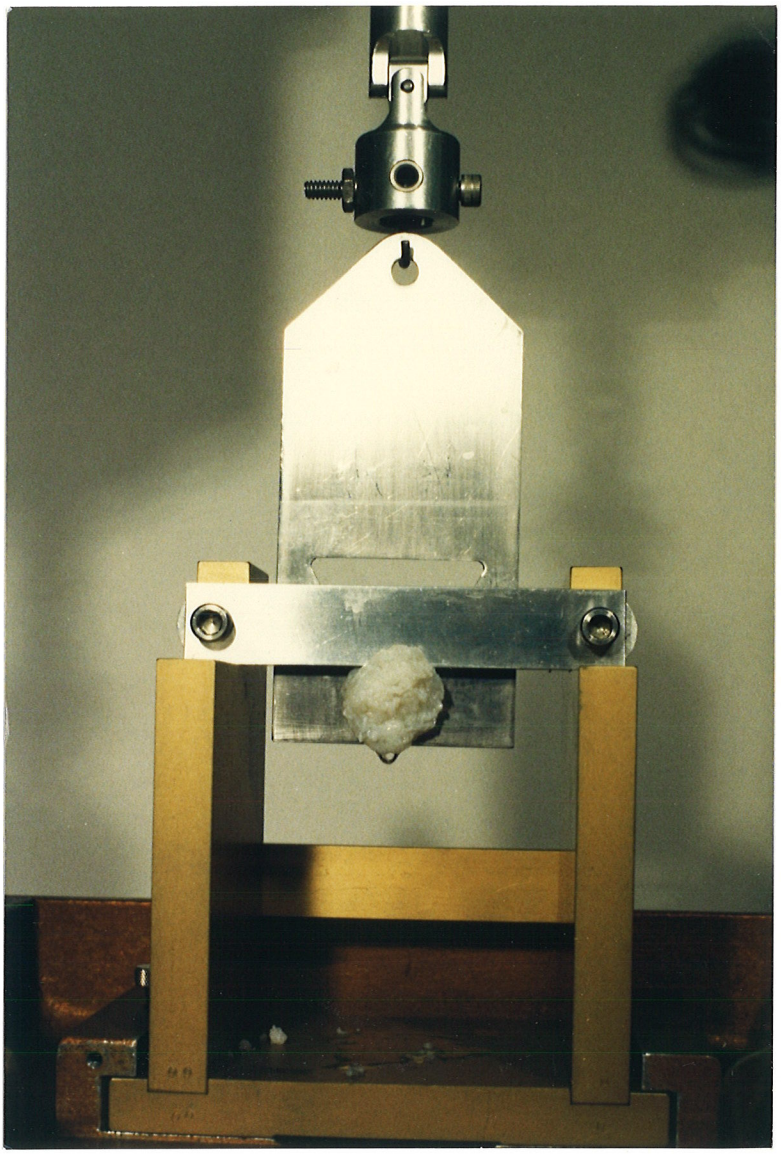

Figure 7. Modified meat cohesiveness apparatus used to assess the cohesiveness of fibre bundles

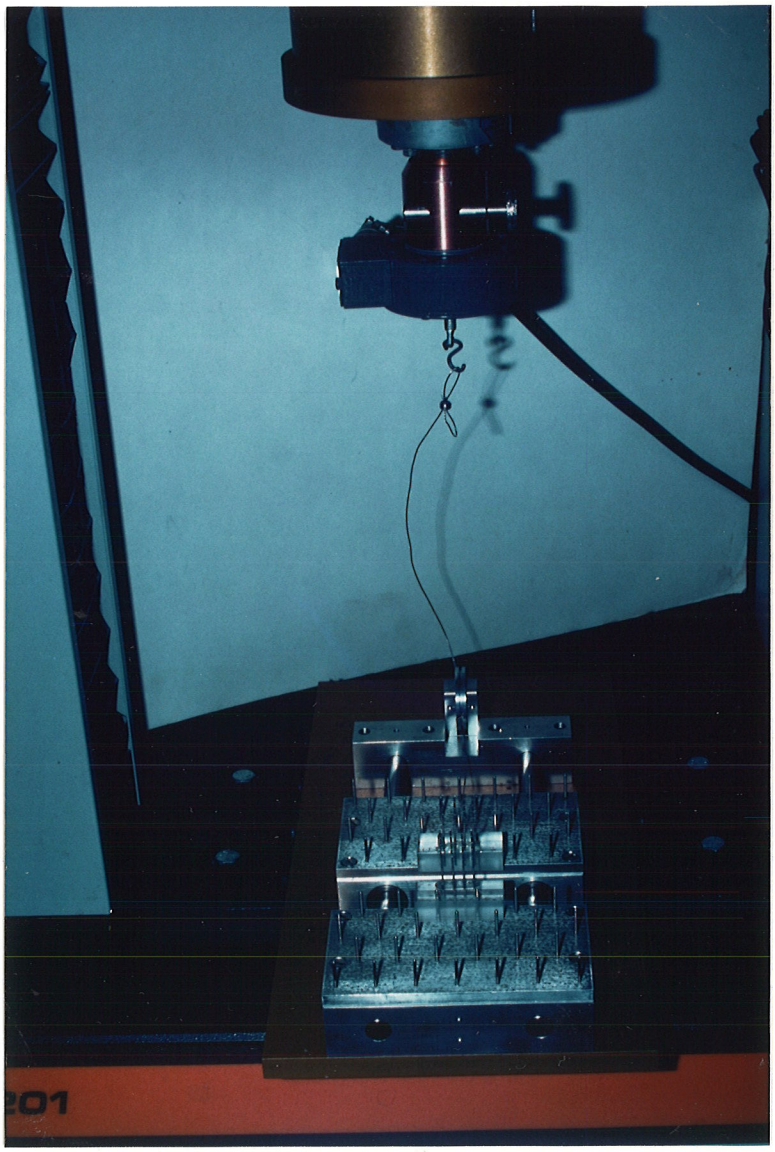
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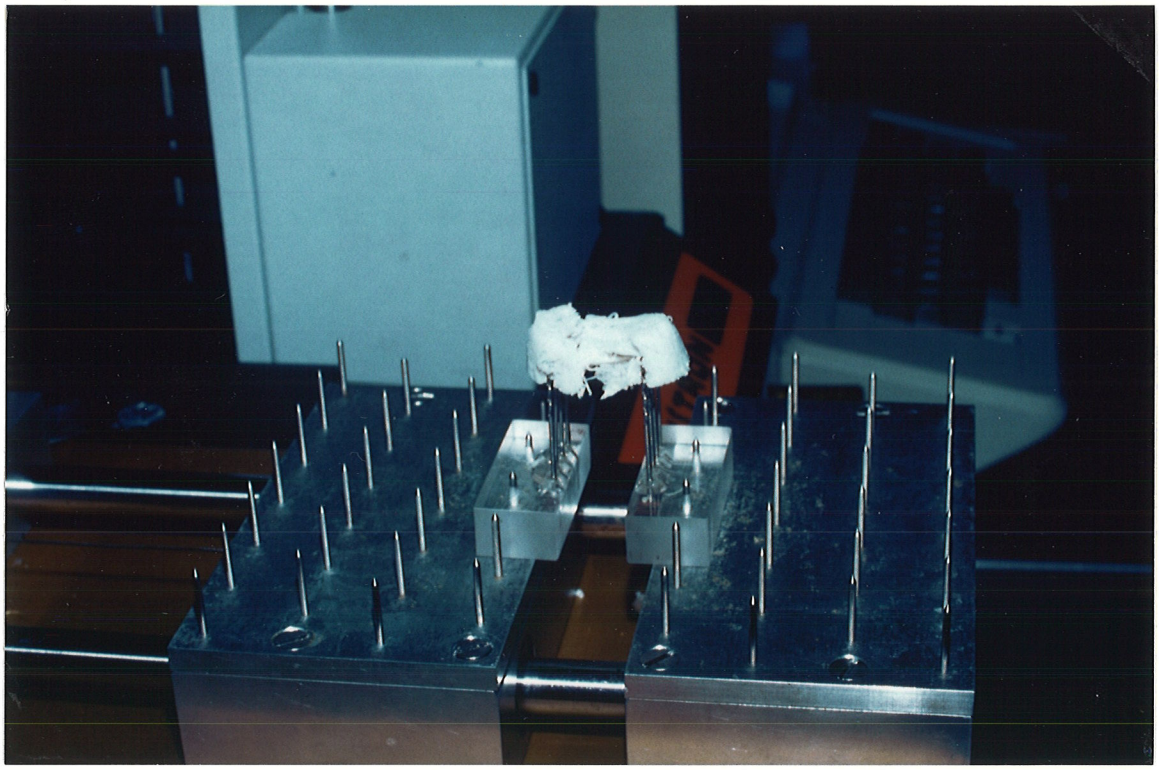
Figure 8. Actual example of a fibre bundle being tested for its cohesive strength



Lebow Load Cell, Model 3108, which in turn was mounted onto the Universal Testing Machine.

3.6. PROTEIN ANALYSIS

The protein content of the following were determined by Kjeldahl analysis (6.25 x N):

i. the fish protein extract before fibre formation

ii. the discarded supernatant of the final centrifugation step

iii. the fibres formed using the LSFFA

3.7. MOISTURE CONTENT

The moisture content of the following samples was determined after drying in a vacuum oven (95°C) for 2 h:

i. fibre bundles exposed to microwave heating

ii. fish protein extract before fibre formation

The percentage of moisture was determined by the dry matter content or the percentage of dry matter in the total wet-based material.

3.8. MICROSTRUCTURE

A limited microscopic analysis was carried out on two products: the fish protein extract prior to fibre formation as well as the fibres formed by the LSFFA. An Amray model 100 A Scanning Electron Microscope (SEM) and a Phillips model 300 Transmission Electron Microscope (TEM) were used.

3.8.1. Preparation of Formed Fibres for SEM

The general procedure for preparing samples for SEM analysis, as outlined by the Food Microstructure Lab,

Agriculture Canada, was as follows:

i. "standard" fixation with glutaraldehyde, but thick and thin fibres were also fixed with ethanolic uranyl acetate solutions in addition to glutaraldehyde

ii. dehydration through a graded ethanol series

iii. frozen in freon and fractured under liquid nitrogen (freeze fractured)

iv. thawed in absolute ethanol

v. critical point dried with carbon dioxide

vi. samples were mounted on aluminum stubs using silver cement sputtered with gold. Fractured surfaces as well as unfractured exterior surfaces were viewed with the SEM

3.8.2. Preparation of Fish Protein Extract and Formed Fibres for TEM

The general preparation of the samples for TEM analysis, as outlined by the Food Microstructure Lab, Agriculture Canada, was as follows:

i. fixed as above ("standard" fixation)

ii. postfixed in osmium tetroxide

iii. dehydrated through a graded ethanol series ending with propylene oxide

iv. infiltrated and embedded in Sparr resin

v. ultrathin sections cut on an ultramicrotome using a diamond knife

vi. sections stained with a methanolic uranyl acetate and Reynold's lead citrate

vii. sections viewed with the TEM

3.9. PHYSICAL COMPARISON OF FORMED FIBRES WITH CONSUMER PRODUCTS

Fresh Alaska King Crab legs, fresh scallops and previously frozen Harbour House Kamaboko were tested for shear strength and cohesiveness.

3.9.1. Preparation

Preparation of the following products was carried out in accordance with the method given by Ismond et al., (1985).

Fifteen fresh scallops were placed into 2 litres of boiling water and allowed to cook for 5 min. The scallops were allowed to cool before their respective diameters were measured.

Three Alaska King Crab legs were placed into 3 litres of boiling water and cooked for 5 min. Once the legs were allowed to cool, the shells were removed. The leg sections were cut into 20 mm and 30 mm lengths and their respective diameters measured.

Ten Harbour House Kamaboko brand "crab legs" were placed into 1.5 litres of boiling water and allowed to cook for 5 min. After cooking, the crab legs were allowed to cool, cut into 20 mm and 30 mm lengths and their respective diameters measured.

3.9.2. Testing

All three products were tested for shear strength and cohesiveness. Testing was carried out following the same procedure as outlined for testing the fibre bundles.

4. RESULTS AND DISCUSSION

4.1. DETERMINATION OF INITIAL DESIGN PARAMETERS

Prior to the design of a fibre forming apparatus, preliminary research was carried out on a bench scale basis to determine the initial design parameters. A Small Scale Fibre Forming Apparatus (SSFFA) was specifically designed and fabricated for this purpose. It was hoped that the research would determine the necessary operating paramaters that were required in order to successfully design and fabricate a proposed fibre forming apparatus. Several key operating parameters necessary to be determined included optimum extruding pressure as well as length and diameter of the extruding holes. Initially, two different SSFFA model designs were constructed and evaluated to determine which method formed superior quality fibres.

4.1.1 Straight Hole Design

No suitable fibres were formed using this particular type of fibre forming apparatus. For the 4 dies tested, it was discovered that extrusion under low pressures (ie. 100-300 kPa) caused a build-up of protein extract at the exit of the die hole. This continual build-up of material at the exit of the hole resulted in the formation of amphorous droplets rather than distinct fibres.

Increasing the extruding pressure resulted in a spraying effect. The excessive pressure caused the

material to be extruded in a spray rather than a solid type fibre. In this instance, the length of extruding hole (ie. thickness) may not have been sufficient to allow the particles of the protein extract to align themselves into an appropiate linear pattern. Castaigne et al.(1983) claimed that the distance over which the protein molecules oriented themselves while passing through the extruding die had a direct relationship with fibre forming ability. Therefore, it appeared the straight hole die was of insufficient thickness to induce proper alignment of the protein molecules necessary for fibre formation.

4.1.2 Hypodermic Needle Design

In this particular design, disposable hypodermic needles were inserted into round aluminum plates to form extruding dies. The use of hypodermic needles offered several advantages:

i. uncut needles provided adequate extruding distance (up to 48 mm) to allow for proper fibre formation
ii. ease of reducing needle length in order to determine the optimum length of the needle die
iii. assurance of a smooth, straight and unobstructed hole
iv. inexpensive and easy method of fibre formation

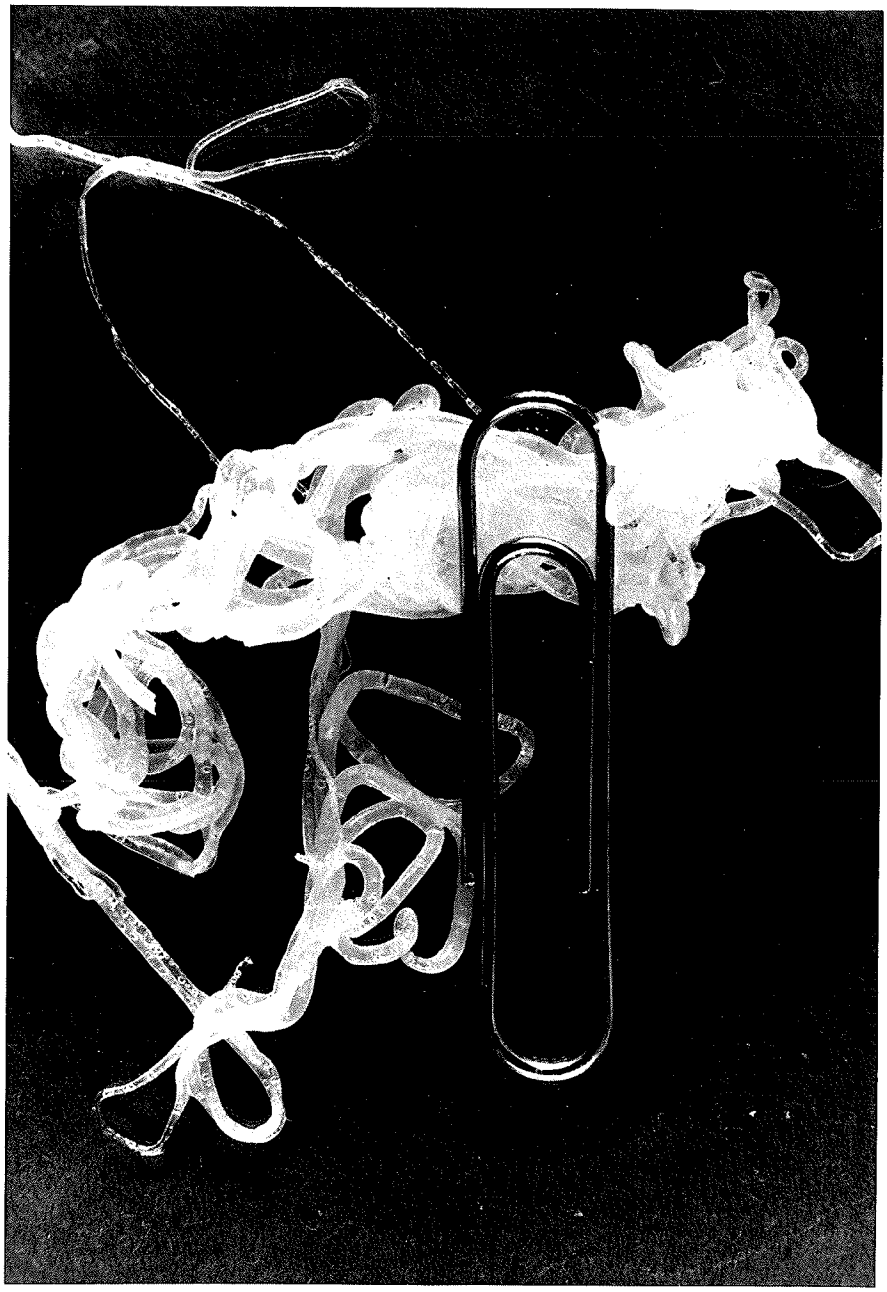
It was found that extruding pressure and needle diameter were critical factors in the formation of acceptable fish protein fibres. Based upon fibre quality, the optimum pressure was found to be approximately 145 kPa

(21 psi). Fibre quality was assessed physically and on a visual basis in terms of strength and stiffness (ie. maximum length of fibre that could withstand its own weight without breaking when removed from the coagulating medium).

The 22 G (0.46 mm diameter) needles produced the most acceptable fibres (Fig. 9). These fibres displayed exceptional strength and stiffness as well as a reasonable visual similarity to real seafood fibres (ie. crab and scallop). Although it may eventually be desired to produce fibres of a smaller diameter, such as with the 26 G (0.31 mm diameter) needle, considerable difficulty existed in overcoming the problem of needle blockage. Minimal blockage occurred using the 22 G needles. The fibres formed using the 18 G (1.07 mm diameter) needles were considered to be too large for eventual application. As well, the larger surface area of the fibres required a significantly longer time to coagulate. Thus, for a fixed coagulation time, the 18 G fibres had a reduced fibre strength and quality when compared to those fibres formed using the 22 G needles.

On a visual basis, the quality of the fibres formed using the 22 G needles did not appear to change when the needle length was changed from 48 mm to 18 mm. Due to the design of the SSFFA, it became impossible to reduce the length of the needles beyond 18 mm; therefore the minimum allowable needle length that produced acceptable fibres could not be found.

Figure 9. Fibres formed using the SSFFA with a hypodermic needle die. Needle is 22 G (0.46 mm) diameter and 18 mm in length. Paper clip shown is 30 mm in length.



4.2 SINGLE SCREW EXTRUDER AS A POTENTIAL FIBRE FORMING APPARATUS

Once preliminary testing of the SSFFA was complete, the results were applied towards the operation of a screw extruder as a potential fibre forming apparatus. An extruding die was designed and constructed to attach onto the discharge end of the extruder. The die consisted of a circular brass plate with forty 22 G disposable hypodermic needles inserted into it in a concentric arrangement.

Once the extruder die was attached to the the pasta extruder, an attempt at forming fibres using a prepared fish protein extract was carried out - however, this proved unsuccessful. During operation of the extruder, it was noticed that the extract would not flow into the fluting of the screw. Unfortunately, the extract remained in the hopper and feed throat area of the extruder and would not feed itself into the extruder barrel. It appeared that the extract did not display sufficient viscosity to allow the screw to pull it into the barrel (as with a dough) but yet ironically, it appeared that the extract displayed too great a viscosity to allow it to flow freely into the fluting of the screw and barrel.

Since it appeared that an alteration of the physical composition of the extract might affect its fibre forming qualities (ie. adjusting the viscosity would likely involve altering the extraction process which would ultimately alter the protein composition), an alternate solution was required. One possible solution involved redesigning and

fabricating a new fibre forming apparatus which would have the capability of handling the physical characteristics of the fish protein extract.

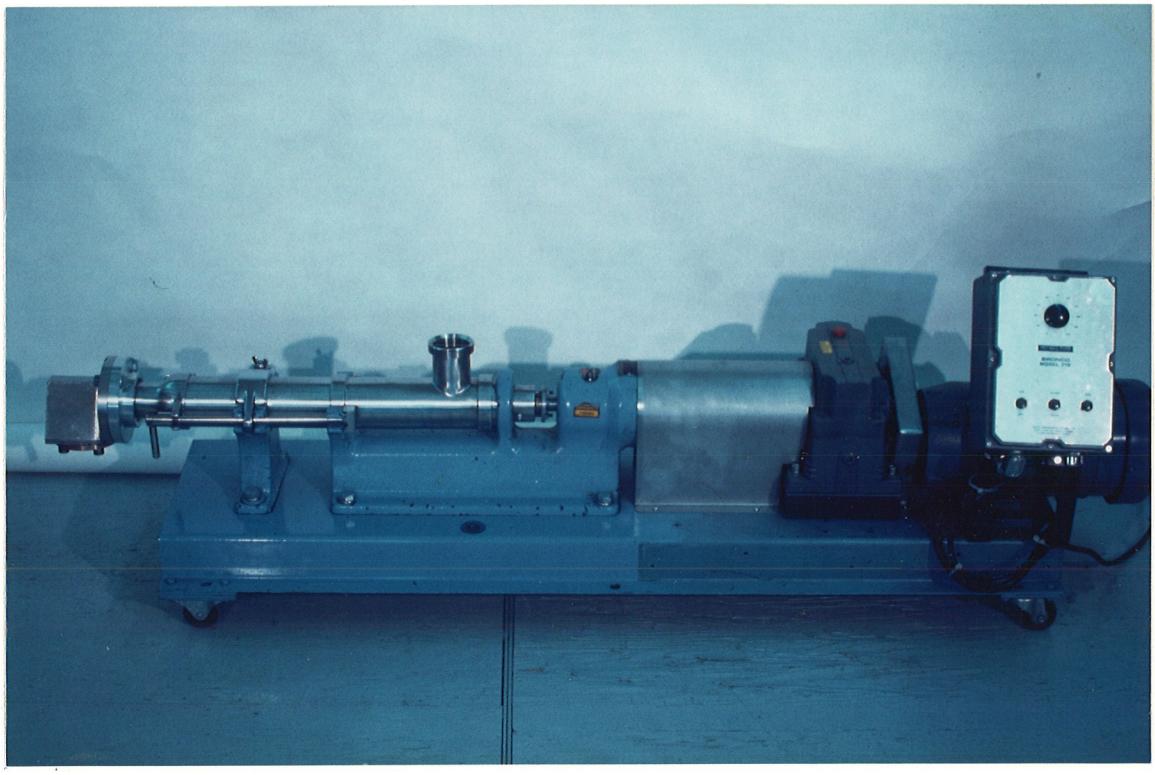
4.3. DESIGN OF A LARGE SCALE FIBRE FORMING APPARATUS (LSFFA)

Based upon the results from the testing of the screw extruder, a search began for a new pumping apparatus capable of handling the physical properties typical of a protein extract. It was decided that a Moyno pump would be particularly suitable for this application.

4.3.1 Moyno Pump Assembly (MPA)

Moyno pumps are designed specifically to handle a wide variety of materials ranging from liquids to very thick pastes. Since the prepared fish protein extracts displayed viscosities typically within the range of 120,000 to 140,000 cps (as measured using a Brookfield viscometer), the Moyno pump was felt to be ideally suited for a material of this particular consistency. A Moyno Pump Assembly was fabricated for the purpose of forming fibres on a continuous basis. The assembly was powered by a 1.5 kW variable speed DC motor which could deliver pressures up to 275 kPa (40 psi)(Fig. 10). The extruding die, originally fabricated for the screw extruder, was modified to enable it to become attached to the discharge end of the Moyno pump. The die still consisted of a circular brass plate with forty 22 G disposable hypodermic needles arranged in a

Figure 10. Moyno pump assembly (MPA; approximate length shown is 2 metres)



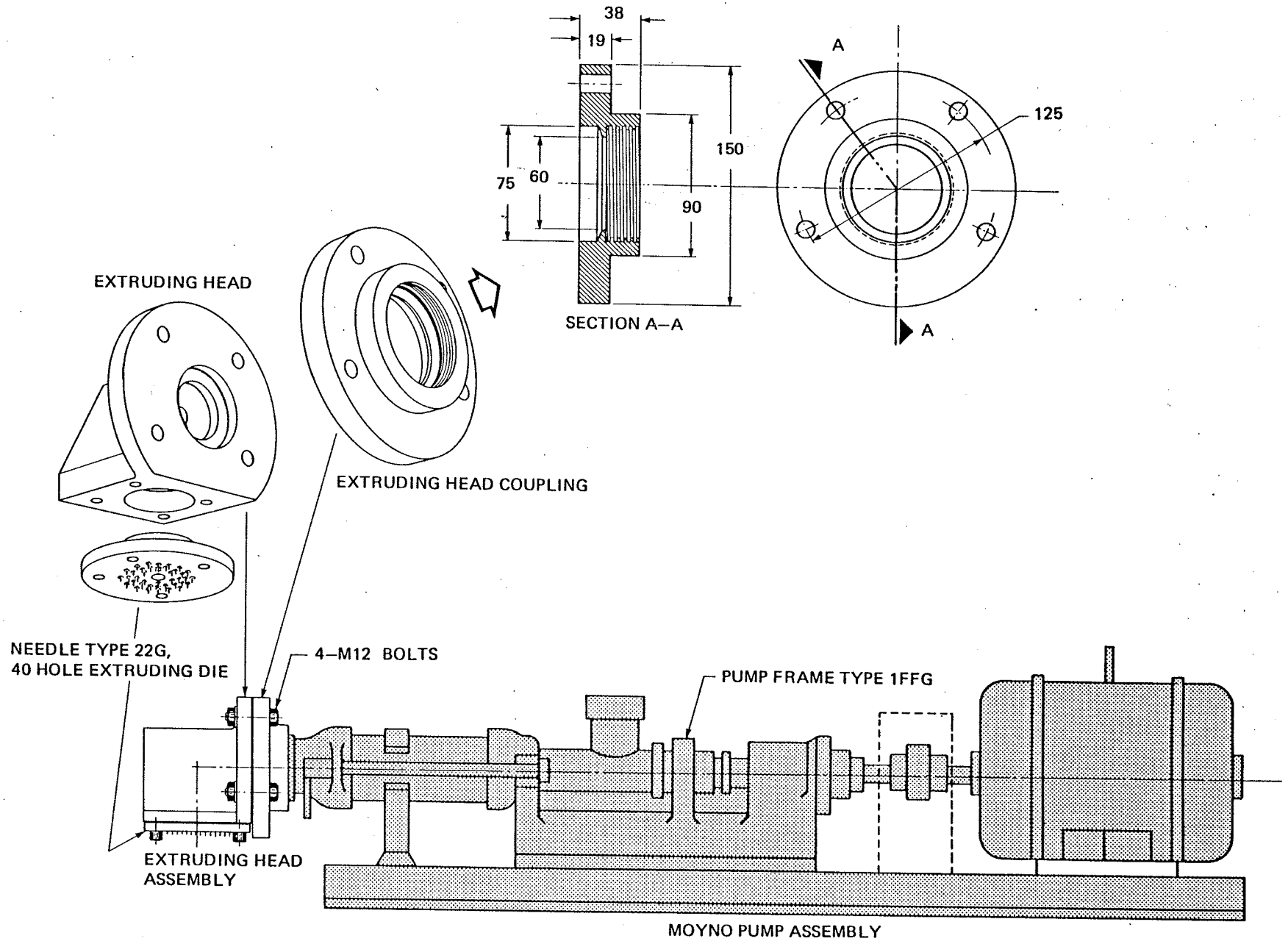
concentric pattern (Fig. 11). Since the length of the needles did not appear to affect the quality of the formed fibres, the needle length was only reduced to 35 mm, to facilitate easier operation of the MPA.

After considerable testing with fish protein extracts, it became evident that the MPA could successfully form fibres on a continuous basis. The optimum operating conditions for fibre formation occurred at an operating pressure of approximately 138 kPa (20 psi), corresponding to a dial reading of 14 on the MPA controller panel with a minimum volume of approximately 1.5 litres of fish protein extract required. Under these operating conditions, the output rate of fibre formation was measured to be approximately 30 mm/sec. These fibres appeared to visually display a sufficient degree of strength and integrity - a necessary quality if the fibres were intended to be used to texturize formulated seafood analogues.

It was discovered that initial volumes of less than 1.5 litres resulted in excessive air being incorporated into the extract during its passage through the MPA. As a result, air pockets were created within the fibres which ultimately reduced the strength of the fibres.

In order to permit the fibre forming process to become a continuous operation, a device was required that would allow the coagulation and removal of the fibres on a continuous basis. At this point in the design stage, the fibres formed by the MPA were simply dropped into a coagulating medium and once a sufficient quantity of fibres

Figure 11. Schematic drawing of Moyno pump assembly showing extruding head and die


38
19
150
75
60
90
SECTION A–A
A
A
125
EXTRUDING HEAD
EXTRUDING HEAD COUPLING
NEEDLE TYPE 22G,
40 HOLE EXTRUDING DIE
4–M12 BOLTS
PUMP FRAME TYPE 1FFG
EXTRUDING HEAD
ASSEMBLY
MOYNO PUMP ASSEMBLY

were collected, the process was stopped. Therefore, a device which would be referred to as a Fibre Conveying Device (FCD), was designed and fabricated specifically for this purpose.

4.3.2 Fibre Conveying Device (FCD)

A Fibre Conveying Device (FCD) was designed and fabricated to carry out three main functions:

1. Coagulate the fibres formed by the MPA
2. Wash the coagulated fibres in an optional cold/ hot water medium after coagulation completed (the purpose of the cold water was to simply wash the fibres while the hot water would have the added effect of further coagulating the fibre)
3. Remove the fibres after washing was complete

Essentially, the FCD consisted of a conveying belt which travelled through two different liquid media. The conveying belt used was 153 mm wide Flat Flex Wire belt and was supported throughout its length by rollers, sprockets and teflon guides (Figs. 12, 13). The operation of the FCD began with fibres, formed by the MPA, dropping onto the wire belting which lay submerged in the coagulating solution. Since the wire belting remained submerged throughout its length in the coagulating medium, it follows that the fibres lying on the belting would also remain submerged while being transported by the wire belting. Once the fibres emerged from the coagulating medium, they

Figure 12. Fibre conveying device (FCD; approximate length shown is 2.8 metres)

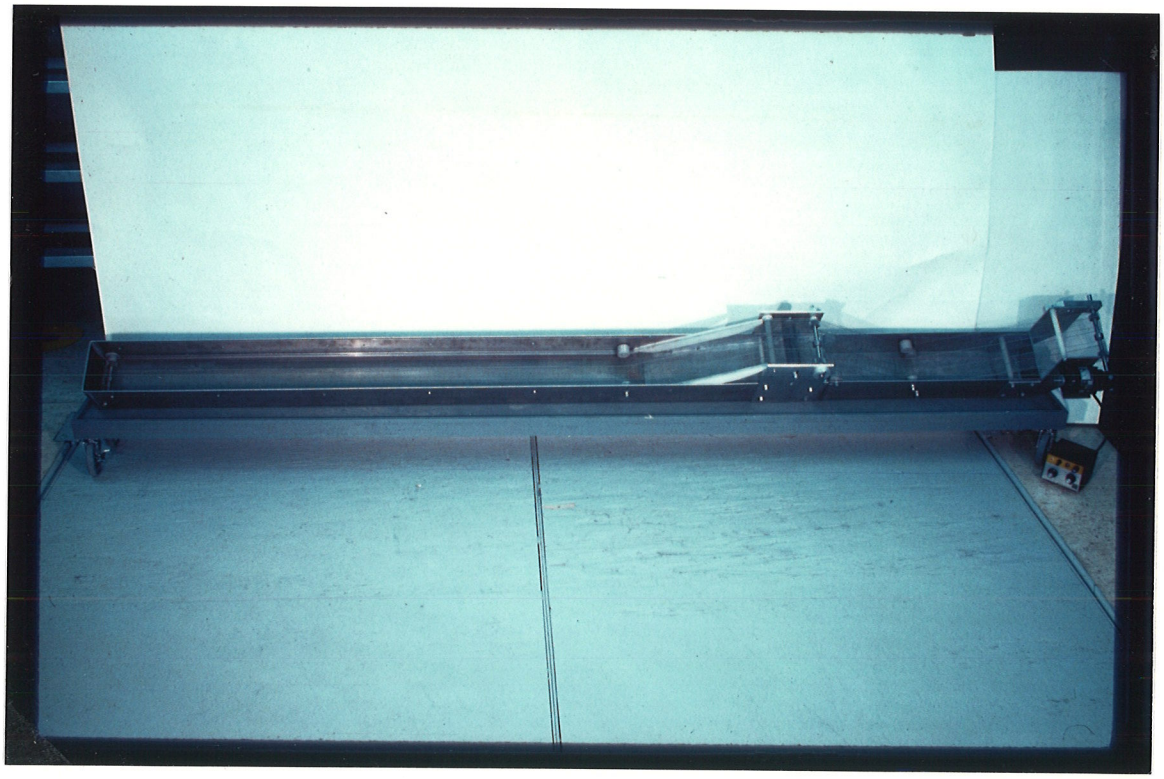
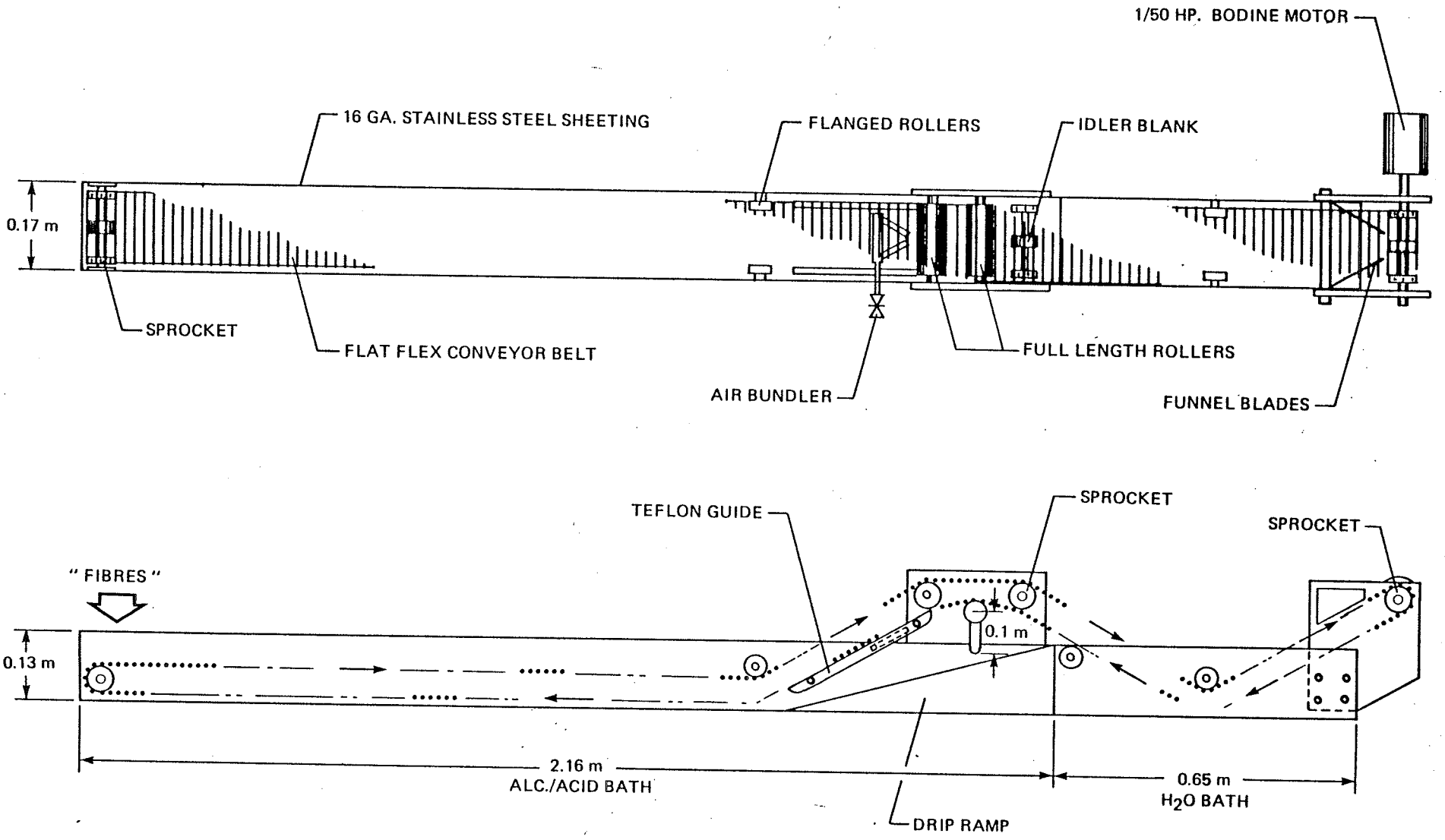


Figure 13. Schematic drawing of fibre conveying device



FIBRE CONVEYING APPARATUS FOR MOYNO PUMP ASSEMBLY

were carried over a "drip" ramp where excess fluid was drained off and the fibres were bundled into a "rope" using a fibre bundler (Fig. 14). In the next stage of the FCD, the rope of fibres passed through a water-wash cycle to remove excess acetified ethanol and then rolled off the FCD into a collecting tray (Figs. 15, 16).

During the design process, the physical dimensions of the FCD were based upon the theory of critical path (ie. the FCD must be capable of processing fibres at the same rate at which they were formed by the MPA). Particular attention was paid to two limiting and dependant factors:

i. the minimum required residence time for the fibres in the coagulating medium

ii. the output rate of the formed fibres from the MPA

Although the surfaces of the fish protein fibres coagulated instantly upon contact with the coagulating medium, additional coagulation time was required to ensure sufficient internal coagulation. This was necessary in order for the fibres to attain adequate strength and to remain as separate structures. Preliminary studies showed that fibres remained independant and separate from each other when submerged in the ethanol-acid medium for 30-40 seconds. When submerged for significantly less time (ie. 20 seconds), the fibres had the tendancy to adhere to each other thereby forming an inseparable conglomerate. Therefore, to ensure proper fibre coagulation, the fibres remained submerged in the coagulating medium for an

Figure 14. Formed fish protein fibres emerging from the coagulating medium onto the drip ramp while simultaneously being bundled into a rope

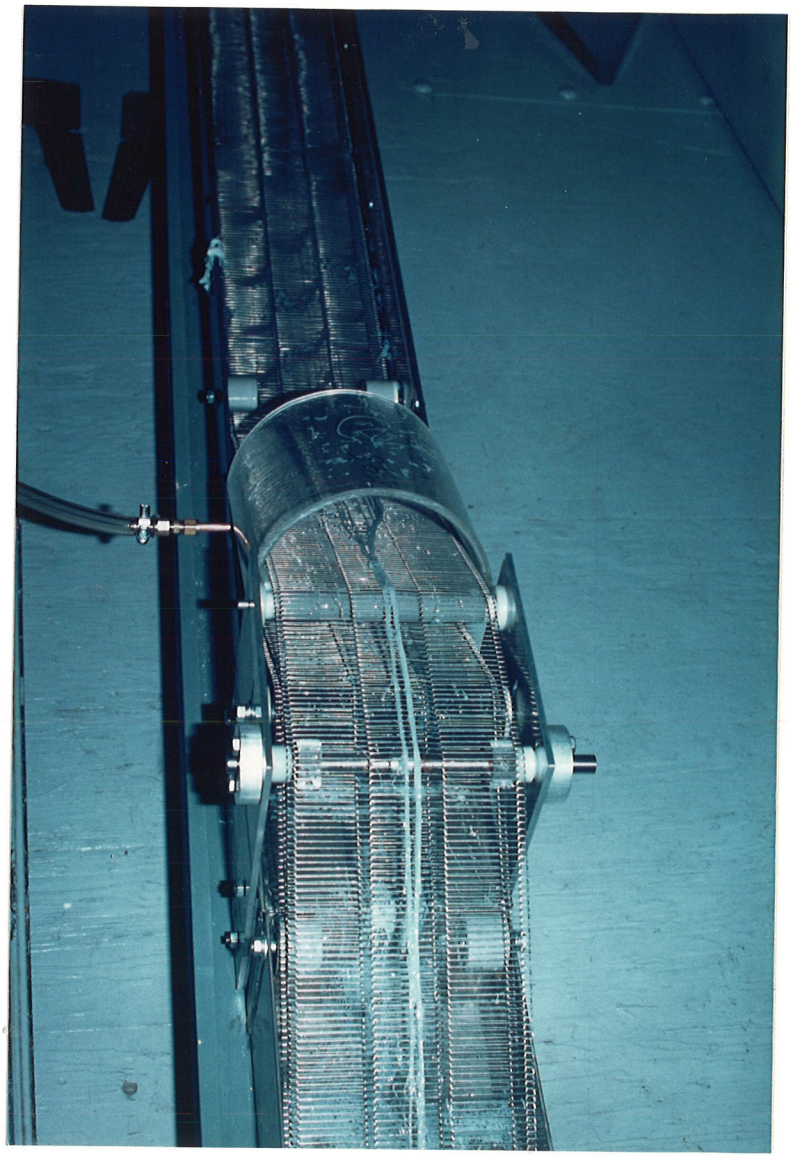


Figure 15. Rope of fish protein fibres travelling through a brief water-wash cycle

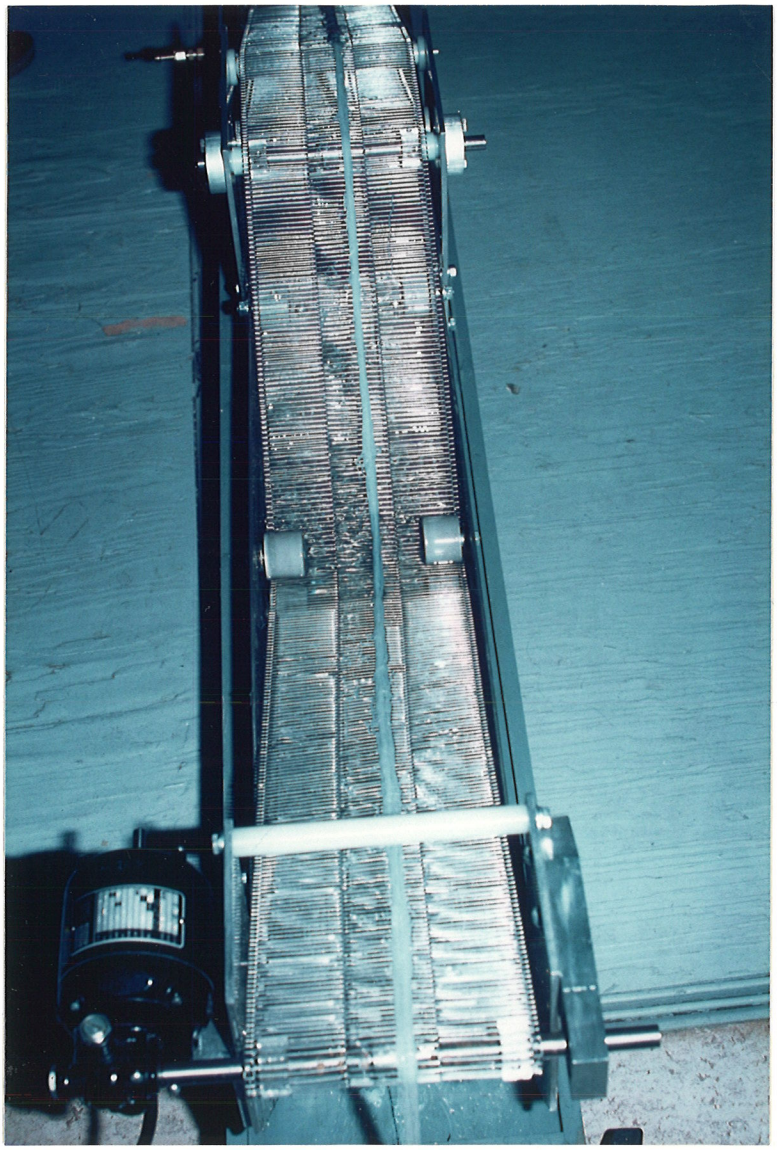
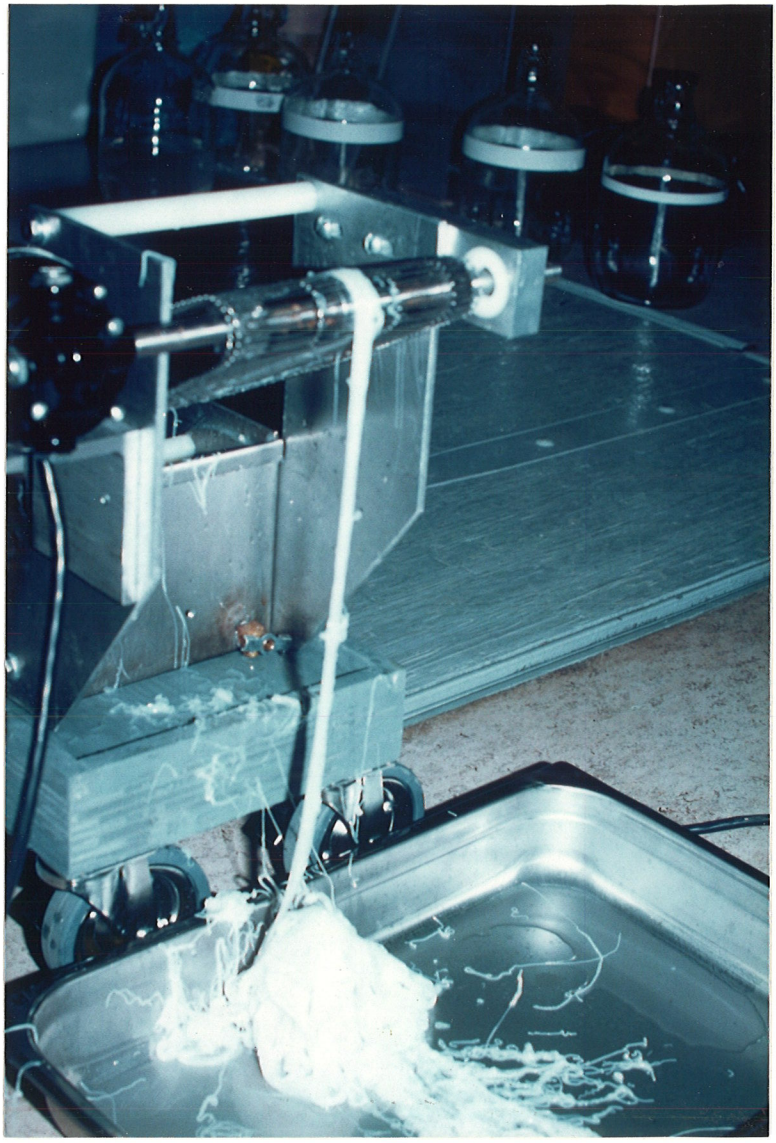


Figure 16. Rope of fish protein fibres rolling off the FCD into a collecting tray



additional 10 seconds, thereby increasing the total coagulation time to 50 seconds. Unfortunately, mathematical models were not used to predict the required coagulation times due to the complexities involved in determining the required coefficients.

Since the rate of fibre output from the MPA was measured to be approximately 30 mm/sec, it became possible to calculate the total length of the coagulating section of the FCD. For example, allowing a minimum residence time of 50 seconds, the required total length was:

30 mm/sec x 50 sec = 1500 mm = 1.5 m

The remaining aspects of the FCD were designed to minimal dimensions (ie. water-wash section) since these were not considered to be as critical (Fig. 13). For example, a simple and quick water wash was felt to be adequate for removal of the coagulating agents still adhering to the surface of the fibre. Taking into account that the maximum angle of incline/decline of the wire belting was 30 degrees (as recommended by the manufacturer), the minimum length of water wash section was calculated to be 0.65 m. This minimum length allowed sufficient clearance of the return line of the wire belting in relation to the walls and bottom of the FCD. Under normal operating conditions, the fibres could have a residence time of up to 10 seconds while in the water wash section; however, residence time could be changed by

either an adjustment in belt speed (if possible) or an increase in water level. It should be noted that the FCD was also fully capable of operating without a water-wash cycle, if required.

After initial testing, it was discovered that the formed fibres adhered to the wire belting at the stage where they were required to "roll" off the FCD. This effect was attributed to the small weight combined with the large surface area of the fibres. A comparatively large fibre surface area combined with a small weight and natural fibre adhesiveness resulted in the fibres adhering to the surface of the wire belting rather than rolling off after the water-wash cycle. To counteract this effect, fibres were bundled into a "rope" before the water-wash cycle. When bundled, the fibre rope contained sufficient weight to enable it to roll off the belting solely through the force of gravity (Fig. 16).

A device, simply known as a fibre bundler, was fabricated in order to provide an effective means of bundling the fibres into a rope. The fibre bundler consisted of a triangular shaped, hollow tubing with a series of 16 holes (0.4 mm diameter) drilled into the two side-tubes. The holes were drilled at various angles such that when connected to a supply of pressurized air, the individual air streams collectively blew the fibres into a rope. The fibre bundler had the capability of bundling a strip of fibres initially 70 mm in width, into a rope of

approximately 15 mm width.

Although the fibres remain intact as a rope, they may easily be separated from each other by simply immersing them into a cool aqueous medium (ie. water). After mild agitation, the fibres separate from each other. Once separated, they may be incorporated into a prepared formulation.

4.4. EVALUATION OF FIBRE QUALITY

After extensive testing of the fibre-forming capability of the LSFFA, there appeared to be several aspects of the the original fibre forming conditions which were believed to have various negative effects on fibre quality . The aspects to be investigated included:

i. removal of the acetic acid present in the coagulating medium. This was not only a residue on the fibres after coagulation but also appeared to reduce fibre quality. Two possible corrective measures included reducing the concentration of acetic acid as well as washing the fibres in water after coagulation.

ii. reducing the alcohol level in the coagulating medium in order to reduce the ethanol residue present on the fibres after coagulation but without excessively jeoporadizing fibre strength.

Since the ultimate goal of this study was to use the formed fibres in various surimi-based seafood analogues, it became important to produce fibres with textural

qualities similar to those of real seafood items. Since any adjustment in the above parameters appeared to affect fibre quality, an investigation was carried out to determine if and how any of these resulting effects could be applied toward the goal of simulating the textural qualities of real seafood items. It was proposed that initial testing involve measuring the tensile strengths of the individual fibres as well as the corresponding effect the above parameters have on tensile strength. It was felt that these results would also aid in assessing which conditions produced fibres of higher tensile strength; this, in turn, should theoretically offer an insight into how resilient each type of fibre would be towards breakage through processing. Unfortunately, when attempting to assess the shear strength of the individual fibres, an unacceptably large degree of error ensued. This was probably a result of the small distance over which the shear strength of the fibre was assessed (ie. fibre diameter of 0.46 mm) combined with a load cell with insufficient sensitivity. As a result, individual fibre quality was assessed primarily on the basis of tensile strength. It should also be noted that implementation of these measures may offer some economic advantage. For example, since the ethanol-based coagulating medium became progressively diluted with water during operation of the LSFFA, rather than replenishing the ethanol after each run, it could be reused until a minimum alcohol concentration

required for fibre coagulation was reached. The minimum alcohol concentration would be determined by the point at which a formed fibre would no longer develop an acceptable tensile strength.

4.4.1. Tensile Strength of Fibres Formed Under Original Conditions

The original conditions of fibre formation involved submersion of the fibre in ethanol with sufficient acetic acid to reduce the pH to 4.2. The fibres remained submerged for 50 seconds and were tested immediately afterwards. Under these conditions, the formed fibres were characterized by a tensile rupture strength of approximately 0.032 N (Table 1). In descriptive terms, the fibres displayed good strength as well as good resistance to deformation. The strength was characterized by the ultimate strength of the fibre or the maximum tensile force the fibre could withstand before rupturing occurred. The resistance to deformation was a measure of the "stiffness" of the fibre (Cernica, 1977). The degree of stiffness was measured as the slope of the best fit relationship of a typical force-deformation curve during tensile loading of the fibre (Fig. 17).

4.4.2. Effect of Washing Fibres Formed Under Original Conditions on Fibre Tensile Strength

Fibres were formed following the same procedure outlined in section 4.6.1. However, after formation, the fibres were submerged in a water bath for approximately

Figure 17. Typical force-deformation curve of unwashed and washed fish protein fibres undergoing tensile loading. Slope of best fit curve indicated by dotted line.

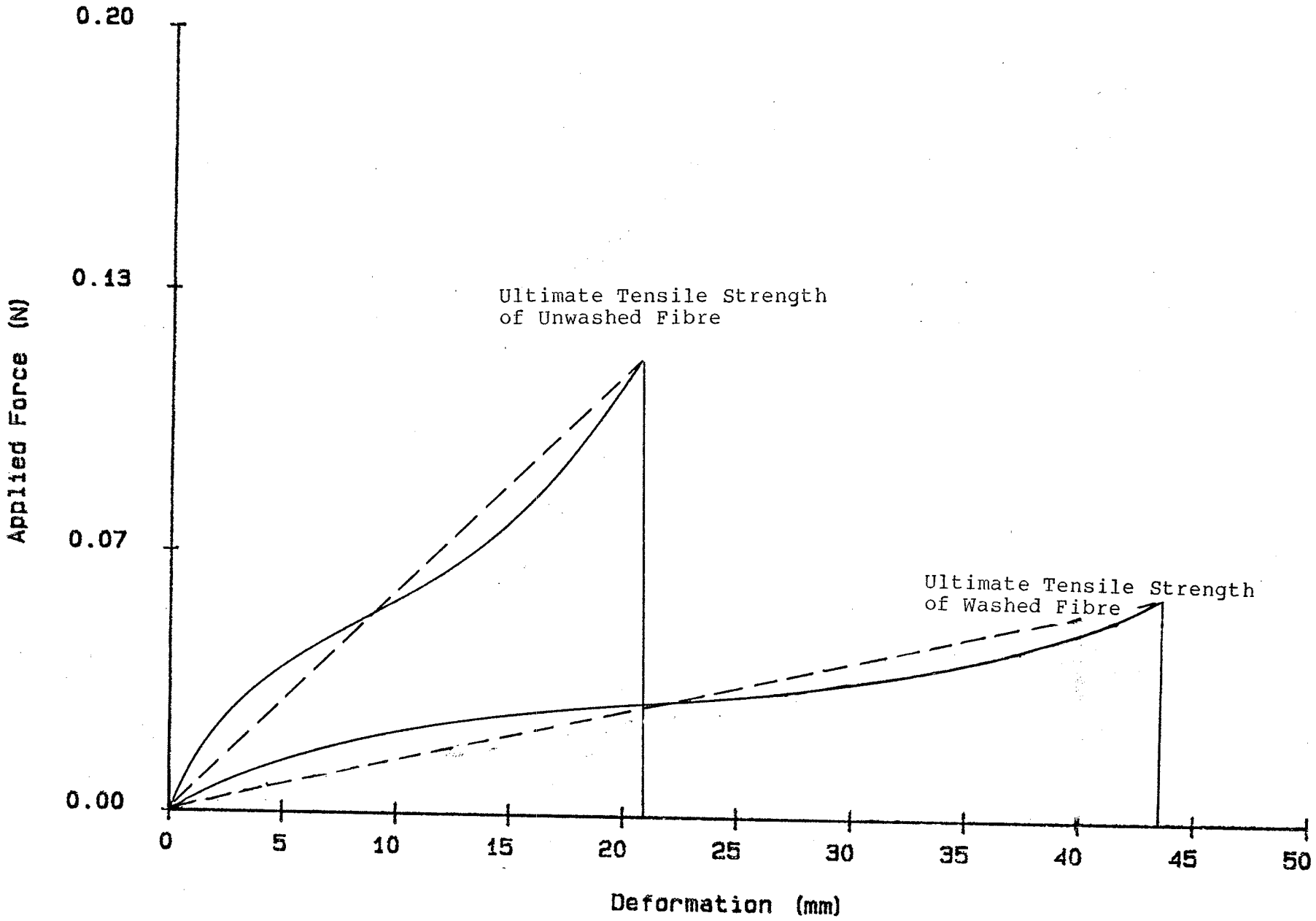

0.20
0.13
0.07
0.00
Applied Force (N)
Ultimate Tensile Strength
of Unwashed Fibre
Ultimate Tensile Strength
of Washed Fibre
0
5
10
15
20
25
30
35
40
45
50
Deformation (mm)

TABLE 1. Tensile strength and stiffness of formed fibres

	Tensile strength (N)[1]	Fibre stiffness(N/mm)[1]
	Mean	Mean
Unwashed	0.032 ± 0.006[a]	0.0010 ± 0.0002[c]
Washed	0.020 ± 0.005[b]	0.0004 ± 0.0002[d]

[1] Mean values followed by the same superscript are not significantly different (Tukey's test, $P<0.05$)

5 seconds before being tested. After this treatment, the mean tensile strength of the fibres was approximately 0.020 N (Table 1). In comparison to the unwashed fibres, the washing step appeared to reduce the tensile strength as well as produce a fibre displaying a smaller degree of stiffness. With respect to fibre stiffness, it was found that the unwashed fibres displayed a mean stiffness of 0.0010 N/mm while the washed fibres had a mean value of 0.0004 N/mm. This weakening effect of the washed fibres could be explained by the partial solubilization of the protein gel structure with exposure to water. The weakening of the gel structure ultimately caused a reduction in fibre tensile strength accompanied with a decrease in stiffness.

4.4.3. Effect of pH of Coagulating Medium on Fibre Tensile Strength

According to Murray and Ismond (1984), an effective coagulating medium contained 95% ethanol with sufficient acetic acid to reduce the pH to 4.2. In further investigations, it seemed that the amount of acetic acid could be reduced without reducing the strength of the formed fibre. This suggested that the ethanol and not the acetic acid, was the most influential constituent of the coagulating medium. An investigation was carried out to determine the minimum level of acetic acid required in the coagulating medium.

The pH of 95% ethanol without acetic acid was 7.3. The tensile strengths of the fibres formed in six different pH media are shown in Table 2. From these results, it appeared that the presence of acetic acid had a negative effect on fibre tensile strength. Fibres formed in a coagulating medium with pH 4.5 appeared to have significantly less tensile strength (0.029 N) than fibres formed in media ranging in pH from 5.0 to 7.3 (0.061 to 0.102 N, respectively).

A typical relationship between fibre deformation and applied tensile force was similar to the force-deformation relationship of the washed and unwashed fibre seen in Figure 17. Fibres formed within the pH range of 6.5 to 7.3 varied in stiffness from 0.0010 to 0.0026 N/mm respectively. As the pH of the media was decreased to 4.5, the stiffness

TABLE 2. Effect of pH of coagulating medium on fibre tensile strength and stiffness.

	Coagulating Medium pH					
	4.5	5.0	5.5	6.0	6.5	7.3
Fibre Tensile Strength[1] (N)	0.029 ± 0.006[a]	0.061 ± 0.013[b]	0.070 ± 0.021[bc]	0.111 ± 0.054[bc]	0.121 ± 0.028[c]	0.102 ± 0.018[bc]
Fibre Stiffness[1] (N/mm)	0.0003 ± 0.0002[d]	0.0008 ± 0.0002[e]	0.0013 ± 0.0001[f]	0.0014 ± 0.0001[f]	0.0008 ± 0.0004[f]	0.0026 ± 0.0003[g]

[1]Mean values followed by the same superscript are not significantly different. (Tukey's test, $P < 0.05$; Appendix Table A).

also decreased to a minimum of 0.0003 N/mm, corresponding to a curve with a decreased slope.

Within the pH range of 4.5 to 7.3, it was decided that the pH of choice would be 7.3. This decision was based upon the inability to remove the undesirable odour of the acetic acid on the fibre despite repetitive washings. Since these fibres were to be ultimately incorporated into a food product, it was desirable to produce nearly odourless and tasteless fibres. Although the fibres formed at pH 6.5 displayed greater tensile strength than those formed at pH 7.3, it was felt that this difference did not warrant the use of acetic acid.

4.4.4. Effect of Reducing the Coagulating Medium Alcohol Level on Fibre Tensile Strength

After establishing that acetic acid was not required for optimum fibre formation, an investigation was carried out to determine the minimum alcohol concentration required for proper fibre formation. As a result of the design of the LSFFA, it was discovered that the wire belt carried water from the water-wash section into the alcohol coagulating section, gradually diluting the concentration of the alcohol medium. It was inevitable that after prolonged operation, the coagulating medium would reach a minimum concentration at which time it would no longer possess the capability of properly coagulating the fibres. Thus, determination of the minimum alcohol level was important from both an economic and product viewpoint. For

example, once a minimum alcohol level was established, it would be possible to determine when the coagulating medium would have to be replaced. This would result in economic savings as well as assurance of proper fibre formation.

Initially, fibres were formed in a coagulating medium containing 95% ethanol. The coagulating medium was diluted with distilled water to give a series of ethanol media ranging from 50 to 95% (Table 3). The maximum tensile strength (0.1022 N) occurred when fibres were formed in 95% ethanol. These fibres appeared to display an appreciable degree of brittleness and consequently broke with minimal fibre deformation occurring. The lack of fibre deformation during tensile loading was confirmed by calculation of the fibre stiffness, or slope of a typical force-deformation curve. Once again, the curves depicting the relationship between fibre deformation and applied force were quite similar to those curves found in Figure 17. For example, the fibres formed in 95% ethanol maintained a degree of stiffness of appoximately 0.0012 N/mm. In contrast, fibres formed in the 50% ethanol medium diplayed a smaller tensile strength (0.013 N) as well as a decreased value of stiffness (0.0001 N/mm). Thus, it appeared that a decrease in alcohol level from 95% to 50% resulted in approximately a ten-fold decrease in tensile strength as well as fibre stiffness. Since there exists a significant difference in tensile strength among fibres formed in 95% ethanol in comparison to those formed in media containing 70% ethanol or less, it would be advisable to maintain the

TABLE 3. Effect of ethanol level on fibre tensile strength and stiffness.

	Ethanol Level (%)				
	95	80	70	60	50
Fibre Tensile Strength[1] (N)	0.102 ± 0.018[a]	0.053 ± 0.0187[b]	0.037 ± 0.009[bc]	0.020 ± 0.007[cd]	0.013 ± 0.005[d]
Fibre Stiffness[1] (N/mm)	0.0012 ± 0.0001[e]	0.0010 ± 0.0001[e]	0.0006 ± 0.0002[f]	0.0004 ± 0.0001[f]	0.0001 ± 0.0001[f]

[1]Mean values followed by the same superscript are not significantly different. (Tukey's test, P <0.05; Appendix Table B).

ethanol content within 80 to 95% in order to maintain maximum fibre tensile strength.

4.4.5. Effect of Storage on Fibre Tensile Strength

An investigation was carried out to determine whether any beneficial textural qualities were acquired by the fibres when stored in the coagulating medium for prolonged periods of time. Fibres were stored for 0, 1, 24, and 48 hours in the original ethanol coagulating medium. After storage, the fibres were tested for tensile strength in both unwashed and water-washed conditions.

The tensile strengths of the fibres were found to increase with increased storage times (Table 4). In the case of the unwashed fibres, the tensile strength increased from 0.029 N after 0 hours to 0.121 N after 48 hours of storage. In comparison, the tensile strength of the washed fibres increased from 0.020 N after 0 hours to 0.049 N after 48 hours of storage. From these results, it appeared that after 48 hours of storage, the unwashed fibres acquired a four-fold increase in tensile strength combined with an six-fold increase in fibre stiffness while the washed fibres acquired a two-fold increase in both tensile strength and stiffness.

When examined visually after 48 hours of storage, the fibres were characterized by a harder texture, having lost much of the softness typical of the unstored fibres. Although the moisture content of the stored fibres was not measured, it was believed that storage in the ethanol

TABLE 4. Effect of storage on fibre tensile strength and stiffness.

		Time of Storage (h)			
		0	1	24	48
Fibre Tensile Strength[1] (N)	Unwashed fibres	0.029 ± 0.006^{a}	0.057 ± 0.013^{ab}	0.080 ± 0.018^{b}	0.121 ± 0.044^{b}
	Washed fibres	0.020 ± 0.005^{c}	0.028 ± 0.003^{cd}	0.046 ± 0.006^{d}	0.049 ± 0.022^{d}
Fibre Stiffness[1] (N/mm)	Unwashed fibres	0.0012 ± 0.0004^{e}	0.0021 ± 0.0012^{e}	0.0052 ± 0.011^{f}	0.0070 ± 0.0015^{g}
	Washed fibres	0.0006 ± 0.0005^{h}	0.0008 ± 0.0003^{h}	0.0012 ± 0.0006^{h}	0.0022 ± 0.0012^{h}

[1]Mean values followed by the same superscript are not significantly different. (Tukey's test, $P < 0.05$; Appendix Tables C and D).

medium effectively removed a considerable portion of the moisture present in the fibre thereby resulting in a drier fibre with a harder texture. As a result, there is doubt as to whether the increase in tensile strength outweighs the changes occuring in fibre texture.

4.4.6. Thermal Effect on Fibre Bundle Shear Strength

The test for shear strength provided a means to measure the "bite" characteristics of the product. For example, a high shear strength would relate to a product with a hard or firm initial bite; in contrast, a low shear strength would relate to a softer, mushy textured product (Finney, 1972). The shear strength of fibre bundles exposed to a variety of microwave radiation levels was measured and later compared with measured shear values obtained from real seafood items. The ultimate objective of this comparison was to identify the thermal process which produced products with textural qualities similar to those of real seafood items.

After adjusting both the pH and ethanol concentration of the coagulating medium, and experimenting with various storage times, it appeared that the maximum tensile strength of an acceptable fibre was approximately 0.102 N (in media with pH 7.3 and containing 95% ethanol). In further investigations, it was discovered that the fibres displayed increased strength when heated. As a result, a study was carried out to determine the effect of heating (ie. microwave) on fibre strength and texture.

Fibres produced by the LSFFA under the original conditions were linearly packed into containers and exposed to five levels of microwave radiation ranging from 0% to 40% of total microwave power (maximum power at 100% corresponding to 600 W). The mean thermal histories of the bundles exposed to the various microwave intensity levels can be seen in Figure 18. From these results, it appeared that the samples subjected to the 30 and 40% power levels followed very similar thermal histories, with each plateauing at a temperature of approximately 90°C after 1 minute. Samples subjected to 10 and 20% power levels displayed different thermal histories, plateauing at temperatures of 40 and 83°C, respectively.

In order to evaluate the effect of microwave heating on the fibre bundles, two different tests were carried out:

Fibre Bundle Shear Strength

The Warner-Bratzler Shear Cell (Fig. 6) was used to measure the shear strengths of the fibre bundles exposed to the various microwave power levels. A comparison of mean sample shear strengths showed that the maximum rate of increase appeared to occur (as a linear relationship) within the range of 0 to 20% (Fig. 19). Beyond the 20% power level, the shear strength began to display a quadratic relationship, plateauing at a maximum shear stress of approximately 20 N/cm^2 (Appendix Table G). From these results, it appeared that an increase in microwave intensity level from 30 to 40% resulted in a very slight

Figure 18. Thermal history of fibre bundles formed using original coagulating conditions and exposed to various microwave intensity levels

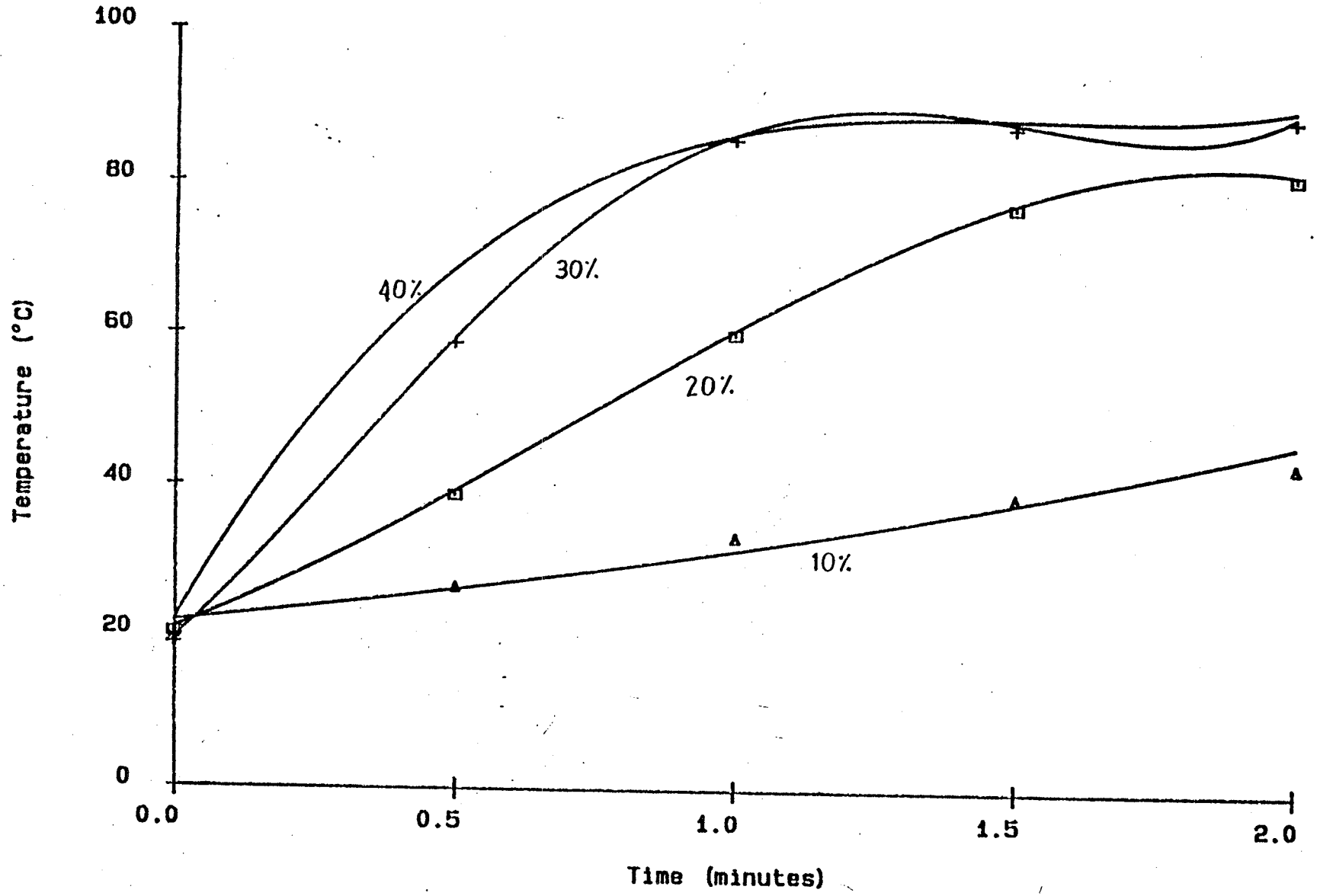
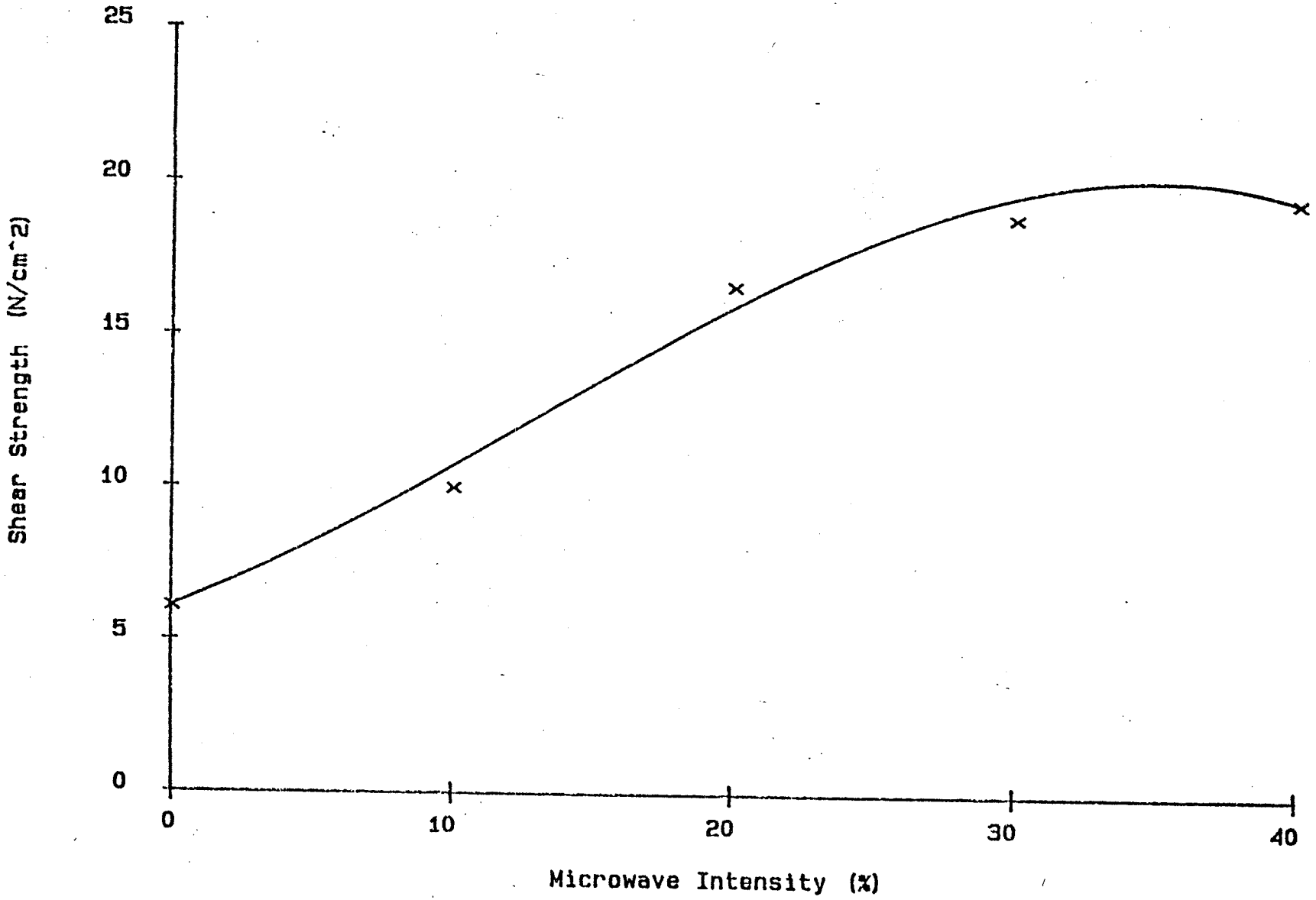

100
80
60
40
20
0
Temperature (°C)
40%
30%
20%
10%
0.0
0.5
1.0
1.5
2.0
Time (minutes)

Figure 19. Shear strengths of fibre bundles, formed using original coagulating conditions and exposed to various microwaveintensity levels for two minute intervals


25
20
15
10
5
0
Shear Strength (N/cm^2)
0
10
20
30
40
Microwave Intensity (%)

increase in shear strength. This assumption also held true for the mathematical equation $y = 5.5 - 0.7x + 0.008x^2$ ($r = 0.9884$), relating microwave intensity (as the independant variable x) to bundle shear strength (as the dependant variable y) as developed through regression analysis. Therefore, it was decided that the spectrum of potentially applicable microwave levels specific for this oven should reside within the range of 0 to 20%.

Bundles that were not subjected to microwave heating (ie. 0% level) were used as control samples. The mean shear strength of the unexposed bundles was approximately 6.0 N/cm^2 (Fig. 19). When exposed to 10% intensity levels, the temperature of the samples rose to approximately 40°C, corresponding to a shear strength of 10.0 N/cm^2. Samples subjected to a 20% intensity level initially displayed a linear time-temperature relationship for approximately 1.5 minutes. After 1.5 minutes, the time-temperature relationship began to plateau, at which point a final temperature of 84°C was reached, corresponding to a shear strength of 16.6 N/cm^2.

Although the final temperature of the samples subjected to the 20% microwave intensity level was only 7°C less than the final temperatures of the samples subjected to the 30 and 40% intensity levels, the thermal histories followed by the samples were quite different. It was believed that the final texture of the samples was directly related to the thermal history influencing it. For example, the samples subjected to 30 and 40% intensity

levels initially displayed a rapid increase in temperature resulting in excessive heating (ie. 90°C) for over 1 minute in duration. The shear strengths of these samples were found to be approximately 19.1 and 19.7 N/cm^2, respectively. Upon examination after heating, these samples displayed excessive dehydration (fibres appeared hard and dry) with a moisture content of 80 and 78% w/w, respectively.

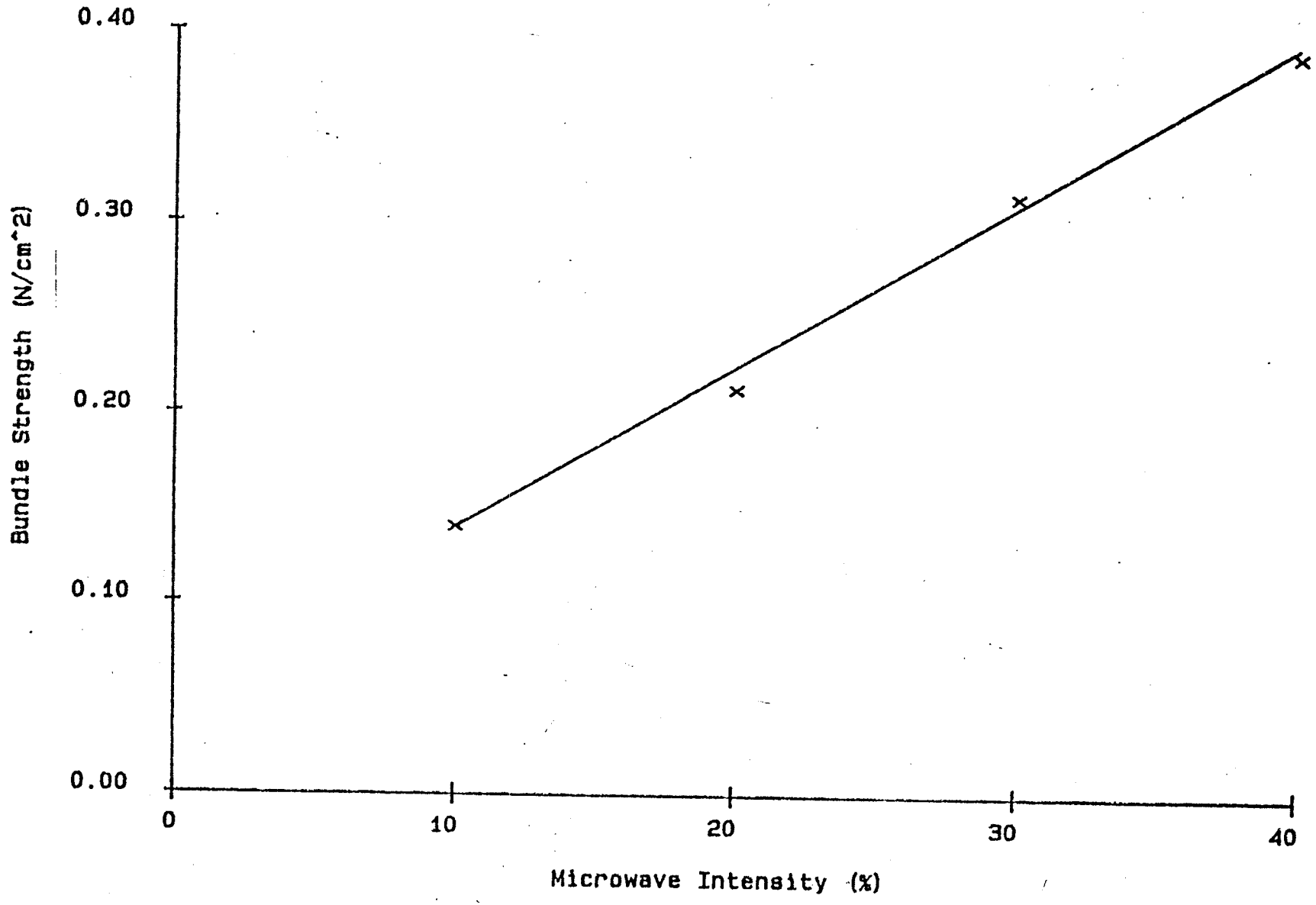
In contrast, the samples exposed to 10 and 20% intensity levels initially underwent a moderate linear increase in temperature with respect to time. These samples were exposed to a maximum temperature of 80°C for less than 30 seconds. As a result, the fibres did not visually appear to display the same degree of hardness or dryness as evidenced with the samples subjected to the 30 and 40% intensity levels. The samples subjected to the 10 and 20% intensity levels were found to have a moisture content of 88% and 83% w/w, respectively. Fibre bundle samples not exposed to thermal heating displayed a moisture content of approximately 93% w/w. It should be noted that although only a 3% difference in moisture content existed between samples subjected to 20% and 30% intensity levels, there existed a considerable but yet unexplainable visual difference in the texture of the two samples. Once again, it is possible that this effect could be attributed to the thermal histories of each particular sample.

Fibre Bundle Cohesiveness

A modified meat cohesiveness test was carried out to determine the degree of cohesiveness displayed by the bundle as a whole. In other words, the amount of force required to separate the individual fibres while in the bundle was considered to relate to bundle cohesiveness. This test was carried out in response to comments from panelists claiming that the fibres within crab-flavoured pollock sections were difficult to separate (Ismond et al., 1985). Therefore, it became a concern that the exposure to microwave heating might impart excessive cohesion within the fibre bundle. As a result, fibre bundles were tested for their cohesiveness after being subjected to a variety of microwave power levels.

The cohesiveness of the fibres within the bundles (or how well the bundle remains intact), was measured by the force to physically pull the samples apart (Figs. 7, 8). It was found that a linear relationship existed between the cohesiveness of the fibre bundles and the microwave intensity level (Fig. 20). Within the range of 10 to 40% of microwave intensity levels, there did not appear to exist an upper limit of measured fibre cohesiveness whereby a further increase in intensity level would result in a decreased value for cohesiveness. The relationship between microwave intensity level (independant variable x) and measured fibre cohesiveness (as dependant variable y) was $y = 0.0515 + 0.0085\,x$, $(r = 0.9956)$, as obtained by regression analysis.

Figure 20. Typical cohesive strengths of fibre bundles formed using original coagulating conditions and exposed to various microwave intensity levels for two minute intervals


0.40
0.30
0.20
0.10
0.00
Bundle Strength (N/cm^2)
0
10
20
30
40
Microwave Intensity (%)

The results from the fibre bundle shear strength and cohesiveness tests were compared in order to assess the range of microwave intensity levels with the greatest potential for eventual application. It became apparent that when fibre bundles were exposed to intensity levels of 10 to 20%, a considerable increase in shear strength and cohesiveness resulted. As well, the fibres did not visually appear to display excessive hardness or dryness. Although a considerable increase in fibre bundle shear and cohesive strength resulted when the intensity level was increased to 30 and 40%, the bundles also acquired an unacceptably hard texture, due to excessive heating and dehydration.

4.5 PROTEIN AND MOISTURE ANALYSIS

Since a sufficient concentration of protein in the extract was of utmost importance for proper fibre formation (Askman et al., 1982), the protein content of the working material was measured at three stages throughout the processing cycle (Table 5). As well, the protein content of the formed fibres was also measured. From the results in Table 5, it appeared that no significant protein loss occurred. Initially, concern was given to the supernatant discarded after the initial centrifugation step. However, since the aqueous supernatant only contained 0.6 to 1.3 mg/ml of protein, this slight loss of protein was considered negligible.

Table 5. Protein and moisture content of working material at various process stages

Product	Protein content[1] % (6.25xN)		Moisture content[1]
Raw Minced Fish	12.7-12.9	(w.b.) (w/v)	83.1-88.8 %
Supernatant after Initial Centrifugation (discarded)	0.6-1.3	" "	N/A[2]
Protein Extract prior to Fibre Formation	4.3-9.3	" "	82.8-93.6%
Final Product-Formed Protein Fibres	5.5-6.9	" "	90.5-90.8%

[1] Values represent ranges from a minimum of 3 samples

[2] Values not available

From reviewed literature, it was suggested that for proper spinning to occur, the protein content of a fish based extract was not to exceed 3 to 4% (w.b.)(Askman et al., 1982). It was claimed that higher protein contents resulted in reduced pumpability and flow. However, since the design of the LSFFA did not incorporate a true spinning apparatus (ie. using a spinnerette with a 100μm diameter), the larger diameter of the extruding holes (ie. 0.47 mm or 470μm), and a larger pumping capacity permitted the use of higher concentrates of fish protein extract.

4.6. ANALYSIS OF SAMPLE MICROSTRUCTURE:

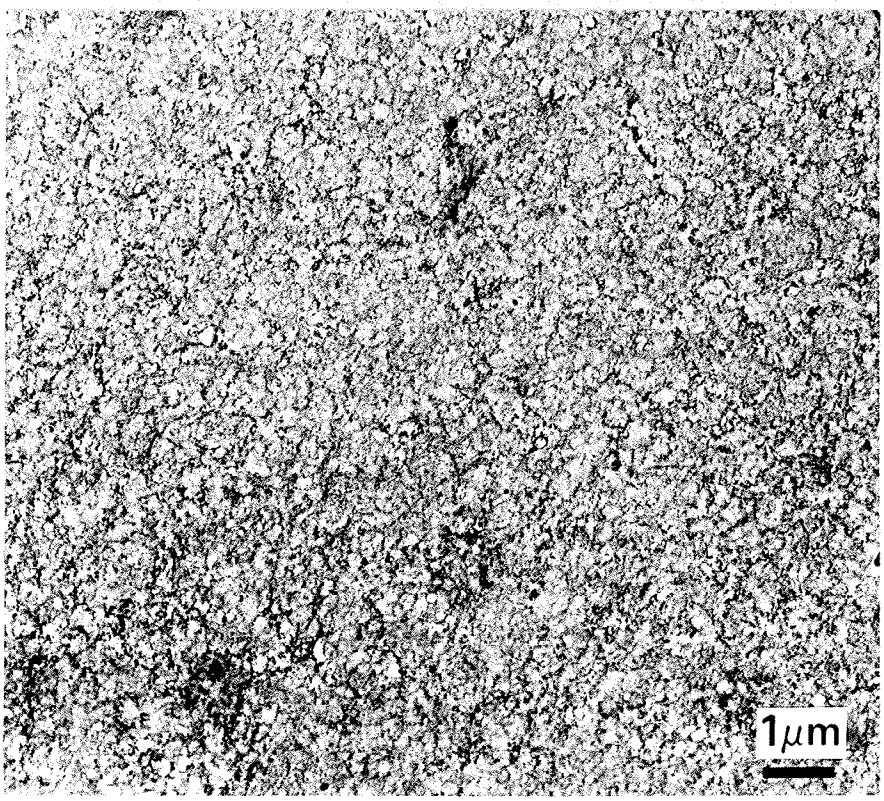
A study was carried out to analyse the microstructure of the fish protein extract both prior to and after its formation into formed fibres. The intention of this study was to acquire an insight into the microstructure of the fish protein material, particularly searching for evidence of a fibrous structure. Although it is possible that the results may not be representative of the entire material, the areas chosen to be analysed were those chosen by a trained microscopist and thought to be representative of the entire sample. In order to insure truly representative results a greater study into the microscopic characteristics of the the samples would be required.

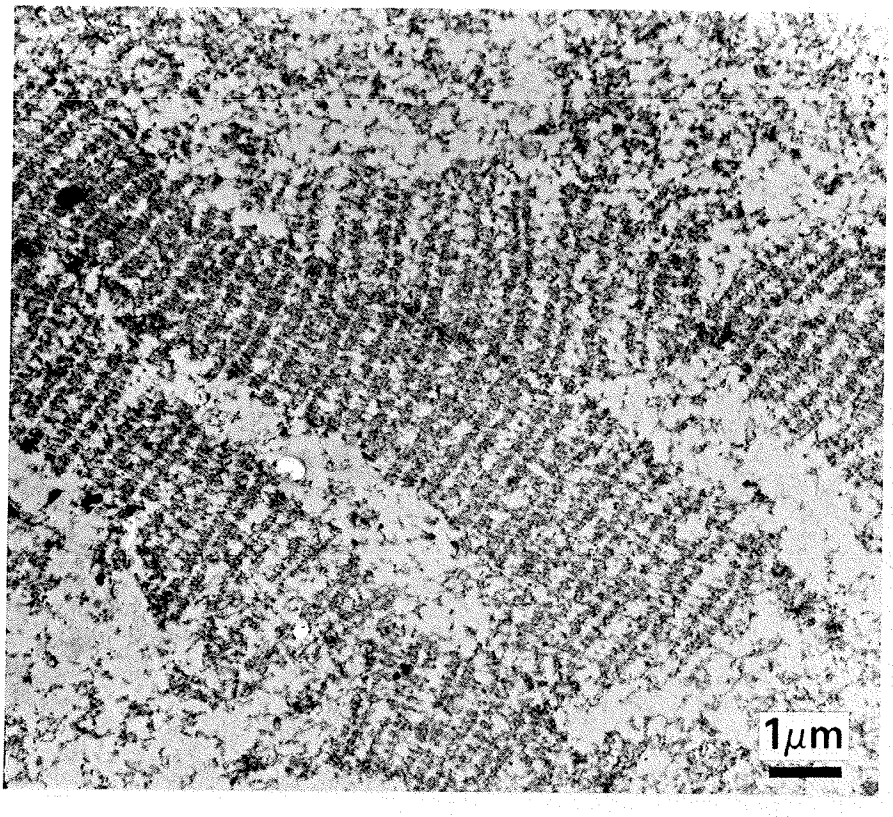
A sample of fish protein extract, typical of that used immediately prior to its formation into fibres, was photographed using a Transmission Electron Microscope (TEM). The sample was characterized as consisting primarily of a gel structure (Fig. 21). According to Cohen et al. (1981), gel structures consist primarily of a network of pores that may or may not be homogeneous in nature. This description typifies the microstructure of the sample shown in Figure 21.

It was found that the gel contained numerous areas of intact muscle fibre (Fig. 22). According to Colombo and Spath (1981) and Vaughan (1979), the presence of muscle fibres was easily detected by the striated pattern of the myofibrils. Inherent in the striated pattern are A zones

Figure 21. Photomicrograph (TEM) displaying gel structure (porous) of fish protein extract prior to fibre formation.

Figure 22. Photomicrograph (TEM) displaying intact muscle fibres (striated pattern) present in the fish protein extract prior to fibre formation


1μm


1μm

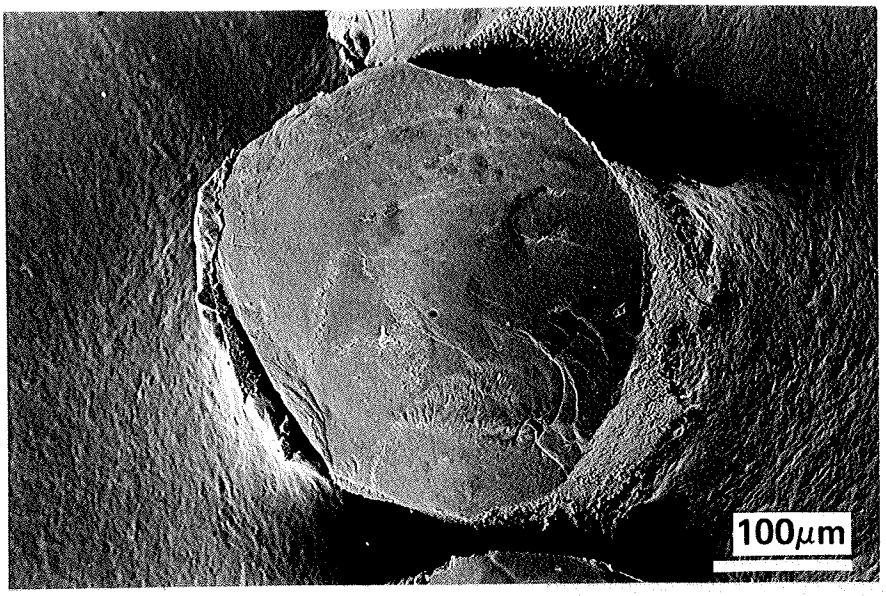
which are shown in the figure as dark bands arranged closely to each other. The presence of the intact muscle fibres indicated either an inadequate homogenization procedure or the limitations of the homogenizer to produce a completely uniform homongenate.

A typical fibre cross-section as seen through a Scanning Electron Microscope (SEM) under low magnification is depicted in Figure 23 A. Under low magnification, it appeared that the fibre was relatively homogeneous in character. However, with greater magnification, it became evident that the fibre was characterized by both a gel structure (ie. relatively porous structure) as well as some intact muscle fibres (Figs. 23 B and C). Since the fish protein extract was similarily characterized, it was not surprising that the coagulated fibre was characterized by both a gel structure as well as the presence of intact muscle fibre.

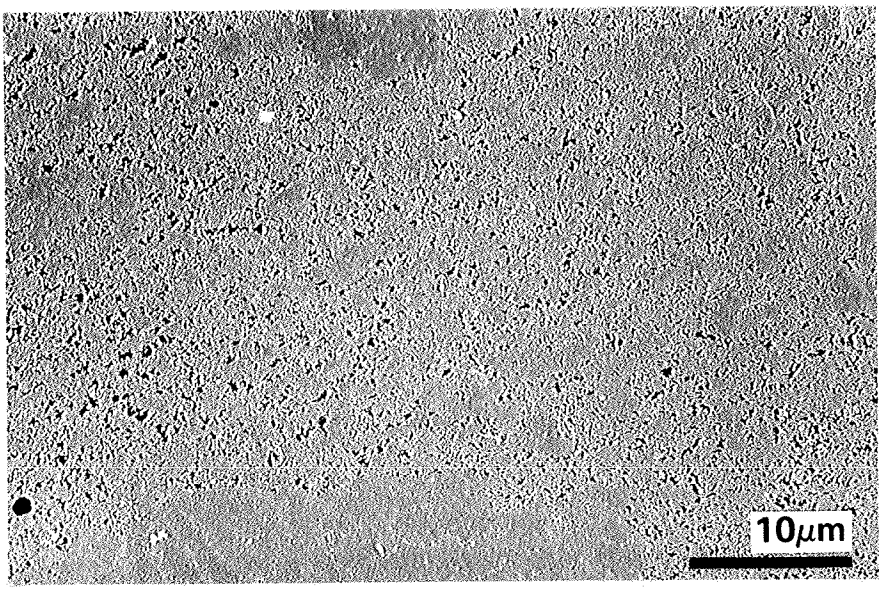
The exterior surfaces of the fibres were analysed using a SEM to determine whether there was any evidence of an induced molecular orientation caused by material flow through the extruding needle. Castaigne et al. (1981), claimed that flow of any highly proteinaceous material through a spinnerette induced a linear molecular orientation of the protein molecules. This induced orientation was thought to increase fibre tensile strength.

Fibres were injected into the coagulating medium using two methods. The first method, assumed to be the

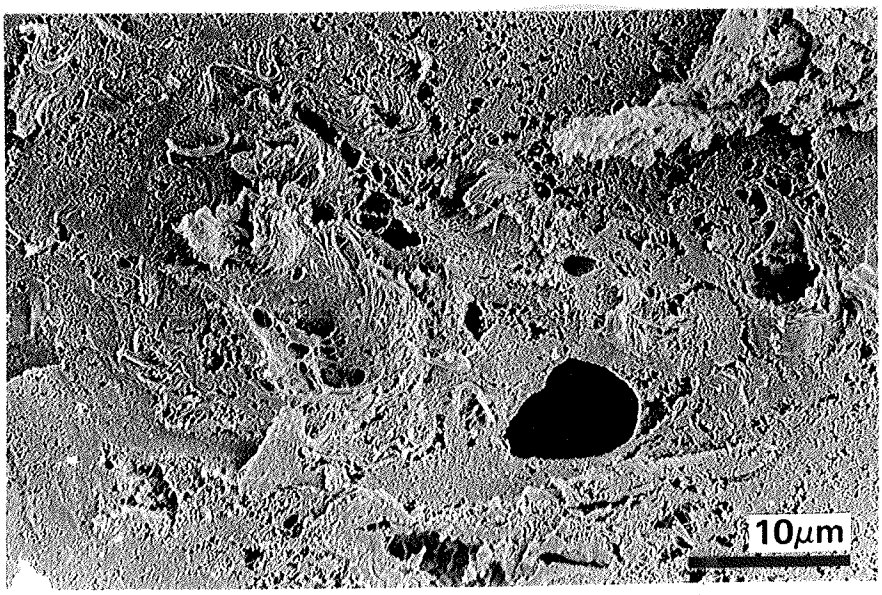
Figure 23. Photomicrographs (SEM) displaying cross-sectional view of a formed fish protein fibre:
A - cross-section of individual fibre
B - typical fibre gel structure
C - intact muscle fibre present in formed fibre



A



B

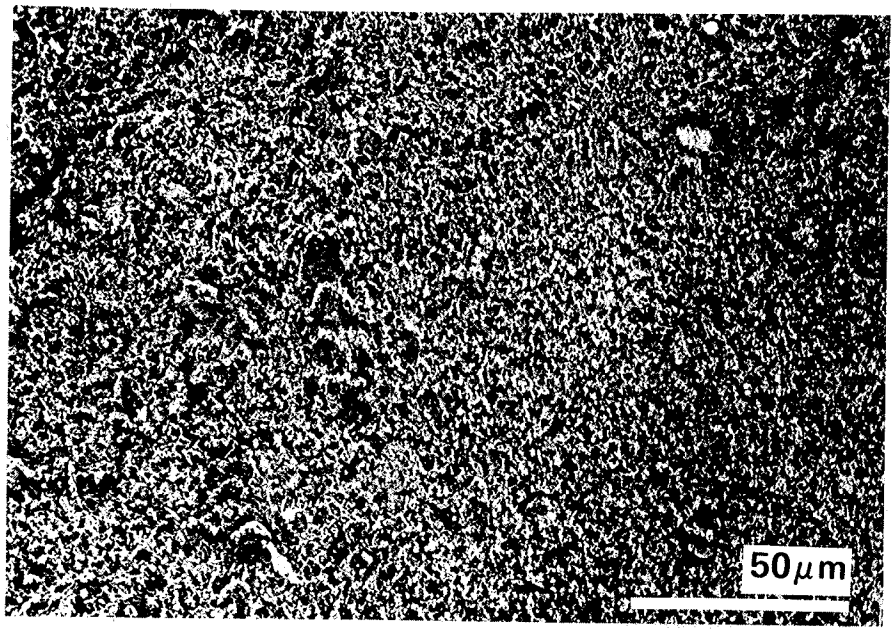


C

conventional method, involved ejecting the fibre out of the needle at a height of approximately 1 cm above the datum of the coagulating medium. The exterior surface of a fibre formed following this method is depicted in Figure 24. The second method involved ejecting the fibre directly into the coagulating medium with the needle submerged. This method formed fibres of significantly smaller diameter (Fig. 25). The effect of the smaller diameter was attributed to the fact that below the surface of the coagulating medium, the pressure at any particular point would include both the atmospheric pressure as well as the hydrostatic pressure of the coagulating medium. In contrast, the first method ejected the fibre initially above the datum of the coagulating medium therefore, only atmospheric pressure is a factor. As a result, when the fibre was ejected from the needle submerged in the coagulating medium (method 2), the increased pressure and the immediate contact with the coagulating medium allowed minimal fibre expansion before coagulation.

It appeared that the fibres formed by initial ejection into atmospheric air (method 1) seemed to have a generally smoother surface in nature. Although no explanation could be found for this phenomena, it would appear that initial exposure to atmospheric air when the fibre exits the extruding needle allows the turbulent or "rough" effect to flatten or dampen out before surface coagulation occurs. Fibres ejected directly into the coagulating medium diplayed a rough surface.

Figure 24. Photomicrographs (SEM) of the surface characteristics of an individual fibre that passed through 10 mm of air layer prior to entering the coagulating medium.


50μm

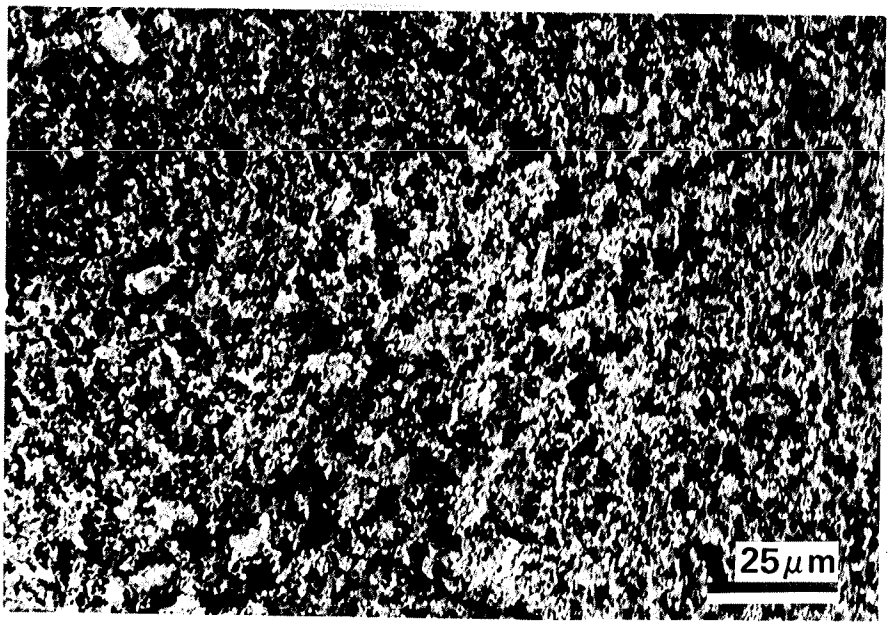
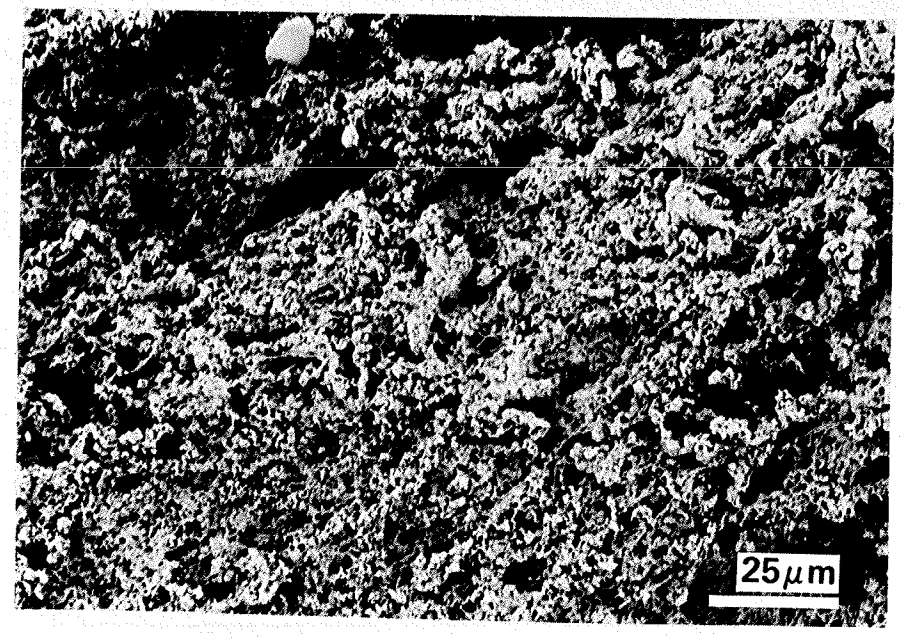

25μm

Figure 25. Photomicrographs (SEM) of the surface characteristics of an individual fibre injected directly into the coagulant


50μm


25μm

Upon examination, the fibres produced by both methods 1 and 2 did not appear to display any evidence of linear orientation occurring on the fibre surface. It should be noted that since the theory, developed by Castaigne et al. (1983), regarding induced molecular orientation is still largely unproven, it is quite possible this phenomenon does not exist during fibre formation. Also, if the stated theory is valid, it is possible that the molecular orientation was simply not detected.

4.7. TEXTURAL COMPARISON OF FABRICATED PRODUCT WITH CONSUMER PRODUCTS

The fibre bundles fabricated in section 3.4.2 were compared with several consumer products for textural similarities. The consumer products included scallops, Alaska king crab legs and crab-flavoured pollock sections (Harbour House Kamaboko). All samples were measured for their respective surface areas, tested and compared on the basis of mean shear strength and cohesiveness (Table 6). According to Figure 19, exposure of the control fibre bundles (ie. 0% microwave intensity level) resulted in a mean shear strength of approximately 5.5 N/cm^2 while exposure to the 10% power level resulted in a mean shear strength of approximately 11.8 N/cm^2. From Table 6, it appears that the fibre bundles exposed to the 10% level

Table 6. Measured textural properties of consumer products

Product	Shear strength (N/cm^2) Mean	Cohesiveness (N/cm^2) Mean
Scallops	4.1 ± 0.7	0.56 ± 0.09
Crab legs	9.9 ± 2.5	0.23 ± 0.10
Pollock sections	4.4 ± 0.9	0.46 ± 0.06
Fibre bundles		
0% level	5.5 ± 0.8	N/A[1]
10%	11.8 ± 1.2	0.13 ± 0.07
20%	16.2 ± 2.1	0.22 ± 0.09

[1] Test could not be performed

have a shear strength comparable to the real crab legs ($9.9\ N/cm^2$). The real scallops and the pollock sections appear to be slightly below the minimum shear stress of the unexposed fibre bundles (ie. 0% level).

A comparison of product cohesiveness showed that the fabricated fibre bundles compared favourably with the real crab legs. Fibre bundles exposed to 20% intensity levels had a mean cohesiveness of approximately $0.22\ N/cm^2$ while the real crab legs had a mean cohesiveness of approximately $0.23\ N/cm^2$. The cohesiveness of the scallop and pollock sections were significantly above $0.22\ N/cm^2$, having values of 0.556 and $0.460\ N/cm^2$, respectively.

In general, it appeared that the texture of the fibre bundles exposed to microwave power levels between 10 and

20% compared quite favourably to real crab legs. Bundles exposed to microwave power levels of 10% acquired similar shear strength values but displayed insufficient cohesive strength. Bundles exposed to 20% levels acquired cohesive strengths similar to those of crab legs but, on the other hand, displayed excessive shear strengths. If the shear strength was interpreted as the "bite" of a food product and the cohesive strength interpreted as the ability of the bundle of fibres to separate from each other, the probable response from a panelist would be the bundle having a similar bite in comparison to crab legs but the fibres within the bundle would separate from each other too easily. Alternatively, bundles exposed to 20% power levels would result in a bundle texture with a similar degree of fibre separation but with a considerably harder bite than crab legs.

The fabricated bundles produced by this method were not comparable to the scallops. For example, scallops typically displayed relatively low shear strengths accompanied by relatively high cohesive strengths. The fibre bundles on the other hand displayed relatively high shear strengths and low cohesive strengths. As a result, any attempt to increase fibre bundle cohesion (thermally) resulted in excessively high shear strength or bite.

It is interesting to note the textural dissimalarities between crab legs and crab-flavoured pollock sections. In regards to the shear strength, the crab-flavoured pollock

sections had a considerably lower shear strength, and therefore should have a correspondingly softer bite. As well, it would appear that the greater cohesiveness of the pollock sections would be perceived as being more difficult to separate which may be attributed to the method of production (ie. rolling "scored" sheets rather than the production of actual fibres) However, actual panelist responses have claimed that in comparison to crab legs, the pollock sections are indeed harder to separate but only a minimal difference in perceived bite was noticed.

In order to increase the ease of separating the fibre bundle without excessively increasing the bite, suitable binders such as starch could be added. Recently, specific gums (ie. Methocel A4M) have been successfully used to increase bundle cohesiveness of fabricated shrimp without excessively increasing the shear strength (Andres, 1984).

5. RECOMMENDATIONS AND CONCLUSIONS

In this study, a prototype fibre forming apparatus was designed which successfully formed fibres on a continuous basis using a prepared fish protein extract. Fibres could be continuously fabricated in sufficient quantity to facilitate their use as a texturizing agent in the formulation and production of surimi-based seafood analogues. The formed fibres were exposed to a variety of conditions including: washing the fibres after coagulation, reducing the ethanol content of the coagulating medium, reducing the amount of acetic acid added to the coagulating medium and storing the formed fibres in the coagulating medium for extended periods of time. The fibres were also packed into bundles and exposed to various level of microwave heating. The investigation into the effect of the above conditions was carried out to determine the overall effect on fibre strength. Since there was considerable concern as to whether the formed fibres could withstand the required handling conditions, it was desired that a fibre of maximum acceptable strength be formed. It was discovered that fibres of maximum strength and quality were formed when no acetic acid was added to the coagulating medium plus the ethanol content of the coagulating medium was maintained as close to 95% as possible. It was also discovered that if the fibres did

not display sufficient strength, they could be exposed to microwave heating which would increase fibre strength.

A series of tests were carried out on various consumer products to determine the textural similarities with the formed fibre bundles exposed to microwave heating. It was found that fibre bundles exposed to a 20% microwave power level (ie. 80 watts), corresponding to a final temperature of 80 °C, compared very favourably with real crab legs. Since higher microwave power levels resulted in overheating the samples thereby forming a hard and dry texture, it is recommended that samples not be exposed to power levels greater than 80 watts. If it is desired to improve the textural characteristics (ie. bite) beyond that attainable through microwave heating, it is recommended that specific binders be used for this purpose.

It is recommended that for a larger production capacity, a microwave heating system be installed onto the FCD. The proposed system could involve the rope of fibres rolling off the FCD and through a wave guide where microwave heating would occur. It is believed that a microwave heating system could be installed that would accomodate a continuous production line.

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TABLE OF APPENDIX

Appendix Table A. Analysis of variance (ANOVA) of the effect of adjusting coagulating medium pH on fibre tensile strength

Source	d.f	SS	MS	F value
pH Level	5	0.056	0.011	12.766 *
Replications	2	1.97E-03	9.85E-04	1.130 ns
Interaction	10	3.74E-03	3.74E-04	0.429 ns
Error	36	0.031	8.72E-04	
Total	53	0.093		

* - significantly different at P<0.05
ns- not significantly different at P<0.05

Appendix Table B. Analysis of variance (ANOVA) of the effect of adjusting coagulating medium ethanol level on fibre tensile strength

Source	d.f	SS	MS	F value
Alcohol Level	4	0.045	0.011	71.887 *
Replications	2	2.178E-04	1.089E-04	0.690 ns
Interaction	8	1.604E-03	2.006E-04	1.271 ns
Error	30	4.733E-03	1.578E-04	
Total	44	0.052		

* - significantly different at P<0.05
ns- not significantly different at P<0.05

Appendix Table C. Analysis of variance (ANOVA) of the effect of storage on unwashed fibre tensile strength

Source	d.f	SS	MS	F value
Storage (h)	3	0.041	0.014	19.818 *
Replications	2	1.500E-04	7.500E-05	0.108 ns
Interaction	6	2.828E-04	4.713E-04	0.681 ns
Error	24	0.017	6.917E-04	
Total	35	0.061		

* - significantly different at P<0.05
ns- not significantly different at P<0.05

Appendix Table D. Analysis of variance (ANOVA) of the effect of storage on washed fibre tensile strength

Source	d.f	SS	MS	F value
Storage (h)	3	9.433E-03	3.144E-03	33.284 *
Replications	2	3.722E-03	1.861E-03	1.971 ns
Interaction	6	1.761E-03	2.935E-04	3.108 *
Error	24	2.267E-03	9.444E-04	
Total	35	0.014		

* - significantly different at P<0.05
ns- not significantly different at P<0.05

Appendix Table E. Analysis of variance (ANOVA) of the effect of microwave power level on fibre bundle shear strength

Source	d.f	SS	MS	F value
Power Level (%)	4	825.496	206.374	100.700 *
Replications	1	6.618	6.618	3.229 ns
Interaction	4	13.436	3.359	1.693 ns
Error	20	40.988	2.049	
Total	29	886.538		

* - significantly different at $P<0.05$
ns- not significantly different at $P<0.05$

Appendix Table F. Analysis of variance (ANOVA) of the effect of microwave power level on fibre bundle cohesiveness

Source	d.f	SS	MS	F value
Power Level (%)	3	0.219	0.073	13.940 *
Replications	1	0.018	0.018	3.369 ns
Interaction	3	0.005	0.002	0.311 ns
Error	16	0.084	0.005	
Total	23	0.325		

* - significantly different at $P<0.05$
ns- not significantly different at $P<0.05$

Appendix Table G. Mathematical models predicting the time-temperature (where x = °C and y = min) relationship within the bundles while exposed to various microwave intensity levels (regression analysis)

		Mathematical Relationship
Microwave Intensity Level (%)	10%	$y = 20.7 + 11.8\ x$, r=0.9928
	20%	$y = 23.5 + 32.0\ x$, r=0.962
	30%	$y = 19.5 + 92.3\ x - 29.3\ x^2$, r=0.9807
	40%	$y = 21.4 + 4.8\ x - 0.07\ x^2$, r=0.9821