UTILIZATION OF LOW VALUE FISHES FOR THE PRODUCTION OF VALUE ADDED PRODUCTS

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2005

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Thesis submitted to the Karnataka Veterinary, Animal and Fisheries Sciences University,
Bidar, in partial fulfillment of the requirements for the
Degree of

DOCTOR OF PHILOSOPHY
IN
FISH PROCESSING TECHNOLOGY

MANGALORE AUGUST, 2005

Dedicated to
my dear wife
Lekshmi

DEPARTMENT OF FISH PROCESSING TECHNOLOGY
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CERTIFICATE

This is to certify that the thesis entitled **"Utilization of low value fishes for the production of value added products"** submitted by **Mr. Dileep. A.O.** for the degree of **Doctor of Philosophy** in **Fish Processing Technology** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, is a record of research work done by him during the period of his study in the University under my guidance and supervision, and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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ACKNOWLEDGEMENT

I wish to express my sincere gratitude to **Dr. B.A. Shamasundar**, Associate Professor, Department of Fish Processing Technology, College of Fisheries, for initiating me this problem and for his valuable guidance and encouragement throughout the course of investigation and in the preparation of this thesis.

I am grateful **to Dr. Keshavanath** (Dean) for providing opportunity and facilities for the study in the College of Fisheries (KVAFSU), Mangalore.

I am very much thankful to **Prof. Nazlin Howell** and **Dr Farah Badii**, School of Biomedical and Molecular Sciences, University of Surrey, UK and **Dr. T.K. Srinivasa Gopal** (Principle Scientist), CIFT, Cochin for providing facilities to conduct a major part of my research work. The help received during the workshop for extrusion process from **Dr. Paul Bartels, Dr. Dick Stegeman** and **Dr. Wouter Jansen** of ATO BV, Netherlands is also greatly acknowledged.

My sincere thanks are due to **Sri. M.H. Bhandary, Sri. R.M. Prabhu, Dr I. Karunasagar** and **Sri. KS Udupa**, who as members of my advisory committee extended the necessary guidance and help during the study. Special thanks are due to **Sri. N.S. Sudhakara, Dr. S Dhananjaya, Dr CV Raju** and **Sri Hanumanthappa** for their suggestions during course work. I also wish to thank all the supporting staff of the Department of Fish Processing Technology especially **Dinesh** and **Ramachandra** for their co-operation. I am grateful to my classmate **Venkatappa**. I extend my thanks to **Basava Kumar, Binsi** and **Prakash** for helping me in practical problems. I am thankful to my friends **Sijo, Dileep, Anil, Babu, Pradeep, Shabari, Jeffu, Pedanna** and **Dipu** for their moral support.

I owe a lot to my **parents, brothers (Pradeep and Sudheer), sister (Deepa), parents-in-law, Anoop** and **my other in-laws (Sujithkumar, Sreeja and Sheeja)** for their love, inspiration and constant encouragement during the period of my study.

The study was carried out during the tenure of a Senior Research Fellowship under a European Commission INCO-DEV project, Brussels and the same is greatly acknowledged.

MANGALORE
August, 2005 (DILEEP.A.O)

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ABBREVIATIONS

BE : Bull’s eye fish
CMFRI : Central Marine Fisheries Research Institute
CSR : Controlled stress rheometer
DSC : Differential Scanning Calorimeter
DVB : Dynamic viscoelastic behavior
FT- : Fourier Transform
G’ : Storage modulus
G’’ : Loss modulus
hr : hour
InGaAs : Indium Gallium Arsenic
KD : Kilo Dalton
KPa : Kilo Pascal
min : Minute
NaCl : Sodium Chloride
Nd.YAG : Neodymium depedyttrium aluminium garnet
PITC : phenylisothiocyanate
RF : Ribbonfish
RPM : Revolutions per minute
SDS PAGE : Sodium Dodecyl Sulphate – Poly Acrylamide Gel Electrophoresis
Tan δ : ratio of loss modulus to storage modulus (G’’/G’)
TEA : Triethylamine
TEMED : N1, N1, N1, N1-Tetramethylene ethylene diamine
T_m : Peak transition temperature
WAC : Water absorption capacity
α : Alpha
β : Beta
ΔH : Enthalpy change
μS : Micro siemens

I. INTRODUCTION

In India, fisheries have always been playing a pivotal role in food and nutritional security of people especially in rural areas. It has significantly contributed towards the improvement of the nutritional status of the populations, since fish has proved as an ideal health food, which is within the reach of the common man (Ghufoorunissa, 2001). Tropical fisheries comprising of several species of fish in varying sizes impose several problems in their handling and preservation. This is one of the reasons for substantial wastage of landed wealth. The fish production in India registered an impressive growth during the last fifty years and in the year 2003-04 the total fish production was 6.67 million metric tons (CMFRI, 2004). The contribution from marine sector accounts for 3.6 mmt and remaining 3.07 mmt is from aquaculture practices. Over the years, the growth in the marine sub sector has slowed down considerably due to dependence on the wild stocks of fish, which are obviously being over exploited. It has been estimated that another 0.6-1.0 mmt can be exploited from unfished/under exploited off shore waters (Somavanshi, 1998). Based on the projected demand of 10 mmt by 2010, the deficit has to be made by other sources. It has been estimated that a large fraction of this resource (nearly 15%) is wasted, either due to discard at sea or deterioration due to spoilage. Hence to meet the increasing demand of fresh fish, one of the strategies is to minimize the wastage and use for human consumption. India has an estimated fish production potential of 8.4 million tons, of which the marine sector potential is 4.0 million tons. In India, there is also the problem of under utilization of by-catch due to lack of means of efficient utilization. The cost effective and efficient utilization of aquatic products demands proper processing and distribution. Demand for fish and fish related products are increasing day by day in India and reduction in post harvest losses can make a major contribution to satisfy this demand.

In order to improve the utilization of underutilized fisheries resources, there is a need to minimize the post harvest losses, develop innovative processing technologies and utilize processing waste for industrial and human use. One such technology, which will be suitable for utilization of low value fish or by-catch, is “extrusion technology”. Use of fish mince with cereals for extrusion process will enable production of shelf-stable products at ambient temperature. Extrusion cooking is used in the manufacture of food products such as ready-to-eat breakfast cereals, expanded snacks, pasta, flat-bread, soup and drink bases. The raw material in the form of powder at ambient temperature is fed into extruder at a known feeding rate. The material first gets compacted and then softens and gelatinizes and/or melts to form a plasticised material, which flows downstream into extruder channel (Chiruvella et al., 1996). Basically an extruder is a pump, heat exchanger and bioreactor that simultaneously transfers, mixes, heats, shears, stretches, shapes and transforms chemically and physically at elevated pressure and temperature in a short time. At times, the extrusion cooking process is also referred as High Temperature Short Time process. Considerable progress has been made in the extrusion cooking technology with the use of cereals, oil seeds and leguminous seeds (Altschul, 1974; Kinsella, 1978).

Extrusion cooking can be carried out either in a single screw or twin-screw extruder. Twin-screw extruders are used for production of a wide variety of food products and the final quality of the end products depends on the characteristics of starch in the cereals and protein ingredient as affected by extrusion process. In extrusion process gelatinization of starch and denaturation of protein is achieved by combined effect of temperature and mechanical shear. The conversion of raw starch to cook and digestible material by the application of heat and moisture is called gelatinization (Chiruvella *et al*., 1996). In the conventional way of gelatinization, water is absorbed and bound to the starch molecules, which results in change in structure. During extrusion the conditions that prevail are high temperature, high pressure, high shear rate and low moisture available for starch may lead to breakdown of starch molecules to dextrins.

The advantages of extrusion process are; a) the process is thermodynamically most efficient b) high temperature short time enables destruction of bacteria and anti-nutritional factors c) one step cooking process thereby minimizing wastage d) destruction of fat hydrolyzing enzymes during extrusion process and enzymes associated with rancidity.

In order to improve the nutritional quality of the cereal based extruded products, addition of fish meat incorporation has been attempted (Choudhury *et al*., 1998). Use of fish mince for extrusion process started in the 1990's basically to utilize underutilized species and fish mince recovered from filleting, canning and surimi operations (Choudhury and Gogoi, 1995). Incorporation of fish meat or hydrolysates along with cereals have been attempted to improve the nutritional quality of extrudates (Choudhury and Gautam, 1999; Choudhury and Gogoi, 1995). The advantages of developing fish based extruded product will help in supplying nutritious and balanced diet to the under-nourished people in the developing countries (Venugopal and Shahidi, 1995). To develop acceptable fish mince-cereal based extruded products, there is a further need to understand protein-polysaccharide interaction so as to evolve appropriate unit operations for enhanced quality products.

In India, during the last decade, with increased efforts to exploit unfished waters, the catches of ribbonfish (*Trichiurus spp.*) and bull's eye fish (*Priacanthus spp*.) have increased considerably. Together the two species accounts for 10% of total marine catch (CMFRI, 2004) and relatively sell at low price. The domestic market for ribbonfish exists both in fresh and salted-dried form. The consumer acceptance of bull's eye fish is yet to pick up and does not attract remunerative prize. Since these two species are available at a lower price and its utilization for extrusion process will perhaps pave way for efficient utilization for human consumption.

The disposal of fish processing waste like skin, bones and viscera is gaining more attention of researchers so as to avoid environmental pollution problem. Many valuable products have been prepared from processing waste and gelatin appears to be more promising in terms of commercial viability. Gelatin is a protein derived from collagen, traditionally obtained from beef and pork skin and bones. Gelatin has been widely used for many years in desserts and meat products as well as for glue production and photographic purposes. Gelatin has played a major role in the development of colloid chemistry and the gel formation in food industry. The surface-active properties and film formation of gelatin have been studied extensively (Montero and Gomez-Guillen, 2000). The largest use of gelatin is in gel desserts. The estimated world usage of gelatin is 200, 000 metric tones per year (Choi and Regenstein, 2000). Gelatin is reported to have characteristic favourable melting properties and mouth feel that are not easily mimicked by polysaccharide thickeners and stabilizers. The physical and chemical properties of gelatin depend on several factors including the method of preparation and the intrinsic properties of collagen. The traditional raw material source for gelatin preparation is bovine and porcine source. With recent outbreak of mad cow disease (Bovine Spongiform Encephalopathy-BSE), the industry is looking for alternate raw material. For different social and religious reasons porcine source as raw material also being limited. Hence utilization of fish skin and bones has a high commercial potential as a raw material for gelatin production.

With this rationale, the present investigation was aimed at evolving suitable technology for extrusion of cereal based fish mince mixture using low economic value fish like ribbonfish and bull’s eye fish. Also, preparation of gelatin from skin of fish (bull’s eye fish) and assessing its properties has been attempted.

The objectives of the present investigation are

- To prepare extruded products of acceptable quality using selected fish species and flours from different sources such as rice, wheat and ragi using twin-screw extruder.
- Optimization of extrusion process – barrel temperature, screw speed, feeding rate and die diameter.
- Quality evaluation of extruded products by instrumental analysis.
- To understand protein-polysaccharide interaction by rheological techniques.
- Preparation of gelatin of acceptable quality from skin of bull’s eye fish (*Priacanthus spp.*) and assessment of rheological properties.

II. REVIEW OF LITERATURE

In order to utilize low economic value fish for human consumption there is a need for development of novel process for production of acceptable, shelf stable and ready to eat products. The preparation of extruded products from cereal-fish mince mixture is gaining more importance world over and as it offers many process variables to get acceptable high quality products. With more and more fish is being diverted for processing, the management of waste generated has to be accorded top priority so as to minimize environmental pollution and also to generate wealth from waste. The present investigation aims at use of low economic value fish like ribbon (*Trichiurus spp.*) and bull’s eye fish (*Priacanthus spp.*) for extrusion process in combination with various cereal flours like rice, wheat and finger millet (ragi). The investigation also aims at utilization of fish waste like skin from bull’s eye fish for the production of gelatin. In this section an attempt has been made to review properties of fish meat constituents, starch from cereal flours, extrusion process technology and properties of gelatin.

2.1. Composition of fish

Fish muscle comprises water, protein and lipids, which make up 98% of the total mass of the flesh. These components play a vital role in the functional properties and nutritional value of the fish. The remaining 2 percent consist of carbohydrates, vitamins and minerals (Ofstad *et al.*, 1996). In general the ash content of most of the fish meat is between 0.5 –1.8% (Sidwell, 1981). The protein content of fish and shellfish varies from 12-20 percent. The proteins from fish and shellfish have been categorized into sarcoplasmic, myofibrillar and stroma proteins (Suzuki, 1981). Myofibrillar proteins constitute 65-70% of total protein and different fractions of myofibrillar proteins are responsible for the textural and functional properties (Kinsella, 1982). The easy digestibility of proteins from fish mainly arises from lesser stroma protein content and nature of myofibrillar proteins (Haard, 1995). Nutritionally proteins from light and dark colored fish meat is almost same (Mukundan *et al.*, 1985). The red meat of fish has more glycine, leucine, arginine and phenylalanine whereas white meat has more lysine, aspartic acid and glutamic acid. Among different fractions of myofibrillar proteins, myosin constitutes 55-60% of myofibrillar proteins (Murray *et al.*, 1993). The eating qualities of fish either in fresh or processed form is mainly related to the properties and behaviour of myosin molecule. The role of other myofibrillar protein fraction like actin, troponin and tropomyosin with reference to eating quality has not been well established (Samejima *et al.*, 1982; Watabe *et al.*, 1982; Kinsella, 1982). Fish contains significant amounts of all essential amino acids, particularly lysine which is a limiting factor in cereals (Garrow and James, 1993). Fish protein can be used therefore to complement the amino acid pattern and improve the overall protein quality of a mixed cereal-fish meat diet.

Fish is one of the most commercially valuable foods, which has been increasingly used because of its high nutritional value due to a high content of proteins and omega-3 fatty acids such as linoleic acid, eicosapentaenoic acid and docosahexaenoic acid (Exler and Weihrauch, 1976; Erickson, 1992, Ackman, 1995). There is evidence that fatty fish and fish oil, which contain the n-3 polyunsaturated fatty acids (PUFA) namely 20:5, n-3 (eicosapentaenoic acid or EPA) and 22:6, n-3 (docosahexaenoic acid or DHA) are beneficial to health (Howell and Saeed, 1999). The proportion of these two PUFA in fish depends on the feeding habits of marine organisms. It is suggested that since most fish feed on zooplanktons, the content of their lipid should be higher in DHA than EPA (Bandarra *et al.*, 2001). Once metabolized, PUFA are eventually transferred through the food web and are incorporated into lipids of aquatic species such as fish. Therefore, increased consumption of marine lipids such as fish has been suggested in order to increase the dietary intake of omega-3 PUFA (Shahidi and Wanasundaram, 1998). Most fish species have different chemical composition, which predominantly depend on their environment, feeding behavior and seasonal variations. Fish fatty acids are the consequences of a balance between food intake and biosynthesis (Bandarra *et al.*, 2001). The abundance of omega-3- fatty acids suggests an additional advantage for the use of the fish species in the formulation of infant foods as they help in the healthy growth and development of the brain, the nervous system and functioning of the eye (Cockburn, 1997). Some of the documented health benefits of the consumption of fish or omega-3 PUFA includes regulation of coronary heart disease and hypertension (Sidhu, 2003; Connor, 2003; Conner and Conner, 1997), stroke (Connell, 1968), cardiac arrhythmia (Sikorski *et al.*, 1976), diabetes (Shenouda, 1980), rheumatoid arthritis (Love, 1988), cancer (Bechtel, 1986) as well as development of nervous system (brain) and vision (Mackie, 1993; Sikorski and Kolakowska, 1994).

2.2. Starch from cereal flours

The importance of starches in the food industry has long been recognized (Dupuy and Laureyns, 2002; Singh *et al.*, 2003). Naturally occurring starch is a mixture of two polymers of D-glucose, a six-carbon monosaccharide (Schuster *et al.*, 2000). These are amylose (linear fraction) and amylopectin (branched fraction). Amylose is essentially a linear polymer of glucose linked through α-1, 4-bonds (Svensson and Eliasson, 1995). Amylose can have a double helix crystalline structure. Schoch (1961) described the helical structure and role of water intervening between the two helixes. X-ray diffraction patterns suggest the helix contains six D-glucose molecules per turn with dimensions such that an iodine molecule can be accommodated within the helix-giving rise to the characteristic blue color of the starch-iodine complex.

In contrast, amylopectin has a highly branched and amorphous polymeric structure containing 4-5% α-1, 6 bonds at the branch points with an average length of a side chain being 20-25 glucose units. Greenwood (1964) observed that individual amylopectin molecules are similar but not identical in their branching configuration. Starch can be hydrolyzed to form a variety of short molecules called dextrins. Dextrins can be formed by the application of heat, shear, enzymes and acids to starch.

Starch molecules are large and their size depends upon the source and the maturity of the plant source. Amylopectin may have a molecular weight in excess of 10^8, making it the largest molecule in nature. In the raw or native state, starch exists in the form of granules. These granules have many shapes ranging from round to irregular and sizes range between 1 and 100 µm (Greenwood, 1964). They are held together with internal hydrogen bonds so that they absorb very little water and the resulting crystalline structure is such that light is refracted during its passage through the granule. When the raw starch granules come in contact with water, they swell and absorb some of the water. Once removed from the water, the granules can be dried and return to their native state. The reversible swelling of starch granules occurs upto a certain temperature called the gelatinization temperature (Atwell *et al.*, 1988; Spigno and Faveri, 2004).

The normal starches (corn, rice, sorghum, potato) contain 17-28% of linear fraction depending on starch species, the balance being branched fraction. However, certain varieties of the cereal starches (the so-called ‘waxy’ types of corn and sorghum) contain only branched fraction, since the plant does not have the genetic ability to form a linear component. At the other end of the scale, certain varieties of pea and corn starches are predominantly linear fraction (i.e., as much as 70-75%) (Schoch, 1961). Microscopically, the starch granules appear to be made up of a series of concentric lamellations. These are more pronounced only in some starches.

Cereal grains and starchy root crops are the major sources of starch. Greenwood (1964) described many of the natural starches and their derivatives commonly used in food formulations. Cereals consist typically of 70% amylopectin and 30% amylose. They form firm gels, which vary with the source of starch. Rice starch is characterized by very small granules but has no other particular unique feature. Modified starches have been developed that preserve the main nutritional features of starch with improved functional properties (Forrest and Cove, 1992; Radley, 1976). The economic value of starch increases according to the degree and type of modification.

The physicochemical and digestibility properties of starches isolated from rice and ragi (finger millet) flours were studied by Madhusudhan and Tharanathan (1995). Gas liquid chromatography analysis of fatty acid methyl ester derivatives of the isolated starch lipid fractions revealed the predominance of C16:0 in rice starch and both C16:0 and C18: 2 in ragi starch. In vitro studies revealed that rice starch to be more digestible in the native state. Shibanuma *et al.* (1996) studied the molecular structures and pasting properties (viscous) of starches from four wheat varieties. The starches showed pasting temperatures in the range of 63.4^{o}-67.6^{o}C.

The granular size of the wheat starch varies greatly. The smallest granules are about 2 microns and the largest about 35 microns in diameter (Olkku and Rha, 1978). Wheat starch is generally considered to be insoluble in water (Knight, 1965). Even industrially produced wheat starch contains some protein. The typical protein concentration of wheat starch was found to be 0.2% (Knight, 1965). According to Jelaca and Hlynka (1971) wheat flour contains about 2% to 3% of water-soluble and water-insoluble pentosans, of which the water-soluble fraction represents only about 0.5% to 0.8% of the total weight of the flour. It has been estimated that pentosans absorb about a third of the total water in normal dough. Granule size ranged from 1.0 to 4.5 μm for rice starch and 1.0 to 9.0 μm for finger millet, ragi starch (Madhusudhan and Tharanathan, 1995), while their shape ranged from spherical to hexagonal / polygonal.

2.2.1. Starch gelatinization

Starch gelatinization and melting is an important phenomenon occurring in various food processing operations because it provides unique textural and structural characteristics for the products (Wang *et al.*, 1989). Glicksman (1969) has given detailed explanation of starch gelatinization. The constituent molecules in the starch granules are held together by hydrogen bondings. When aqueous suspensions of starch granules are heated, a temperature is reached at which hydrogen-bonding forces are weakened to the point where water can be absorbed by the granules. At this temperature, called the ‘initial gelatinization temperature, the granules swell tangentially and simultaneously lose their birefringence (Atwell *et al.*, 1988; Donovan, 1979). This phenomenon starts at the hilum or botanical centre of the granules and spread rapidly to the periphery. Gelatinization begins in the intercellular areas where the hydrogen bondings are weakest. It occurs in different temperature ranges for different starches. Values for wheat starch are: initial gelatinization temperature, 58^{o}C; midpoint, 61^{o}C and end point, 64^{o}C (Olkku and Rha, 1978). As the temperature of the aqueous suspension is increased above the gelatinization range, hydrogen bondings continue to be disrupted, water molecules become attached to hydroxyl groups and the granules continue to swell. With the continued swelling of granules, starch particles, which have become fully hydrated separate themselves from the intricate micellar network and diffuse into the aqueous medium (Glicksman, 1969).

Gelatinization is the major transition of starch molecule during thermal processing. It involves three steps and general scheme of gelatinization process is given in Fig. 1. Initially the water addition breaks up the amylose crystallinity and disrupts its ordered structure. As the process continues, the starch granules noticeably swell, increasing their volume by 25-30 fold and disrupts the granule crystallinity. As more heat and water are added, the amylose begins to diffuse out of granules and the starch is no longer in the reversible granular state (Donovan, 1979; Wang *et al*., 1991). As the gelatinization process continues, the granules collapse and more and more water molecules attach themselves to the exposed hydroxyl groups on the starch chain. A colloidal gel structure results with the amylose supporting the collapsed granules consisting mostly of amylopectin.

Characteristic gelatinization temperatures exist for different types of starch. In reality, the gelatinization temperature is a range of temperature over which the gelatinization process occurs. Miller *et al*. (1973) have taken photomicrographs of starch

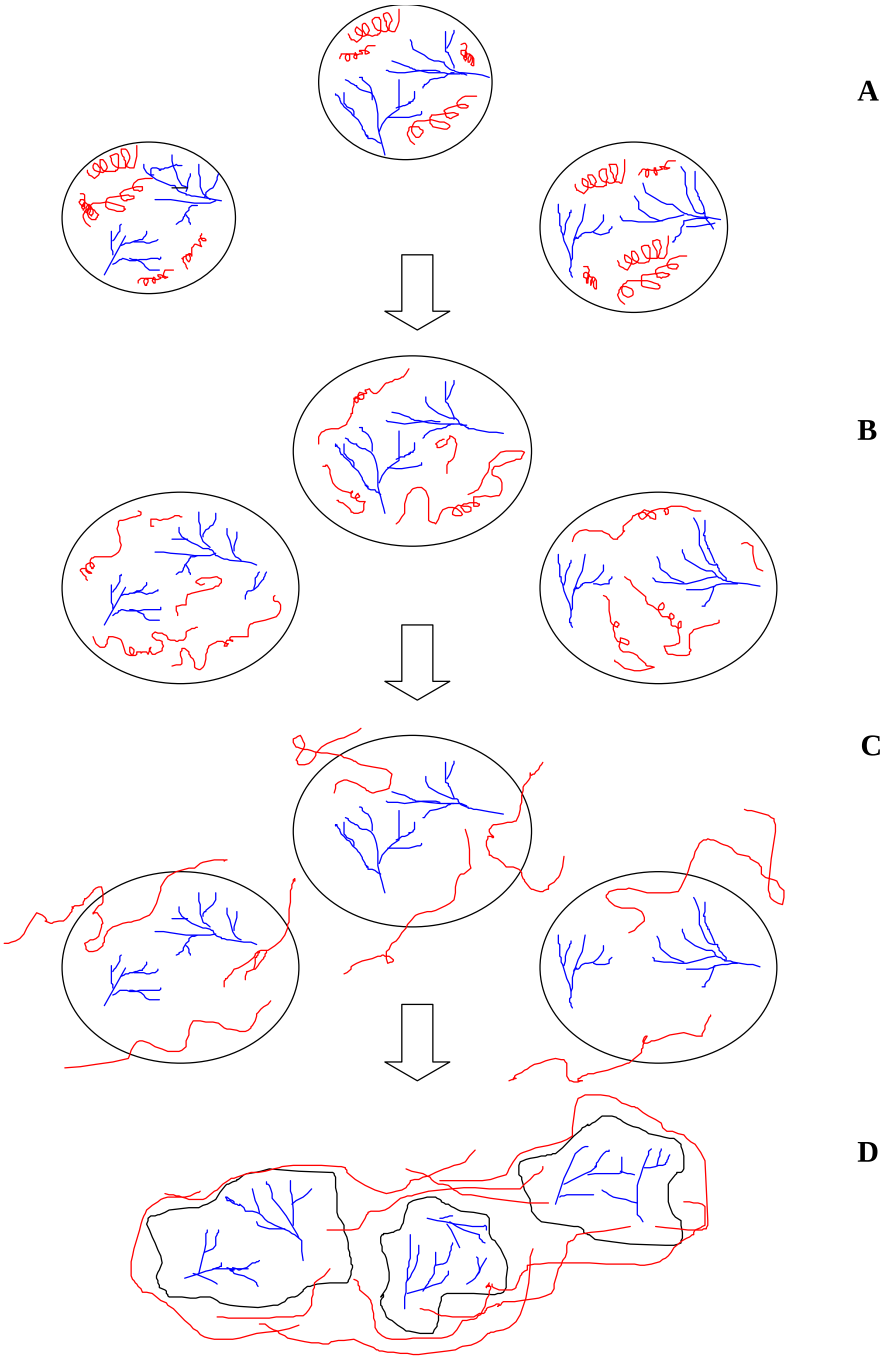

Fig. 1. Mechanism for starch gelatinization (From Remsen, C.H., and Clark, J.P. J. Food Proc. Eng., 2(1), 39, 1978)

A: Raw starch granule made up of amylose (helix) and amylopectin (branched).

B: Addition of water breaks up amylose crystallinity and disrupts helices. Granules swell.

C: Addition of heat and more water causes more swelling. Amylose begins to diffuse out of granule.

D: Granules, now containing mostly amylopectin, have collapsed and are held in a matrix of amylose forming a gel

during gelatinization. The picture clearly showed that granule swelling is not sufficient for high viscosities of starch/water colloids. Very little information is available on the gelatinization of starch at moistures less than 50%, the range where extrusion processing is practiced. Based on stoichiometry, with one water molecule bound to each available hydroxyl group on the starch, a minimum 25% moisture level would be required. Low levels of water are sufficient to interact with starch in extrusion ingredients to plasticize the mass and form dough.

Apparently the heat of gelatinization increase in the following order: cereal < root < tuber starch. At the molecular level, the heat of gelatinization involves the breaking of hydrogen bonds existing between starch molecules and the formation of new bonds with water to give a less ordered structure and increased entropy. Gelatinization of starch is basis for many types of food production. Food industries make use of several types of starch. Tuber starches such as potato and tapioca, and grain starches such as corn and wheat, are the most commonly used (Olkku and Rha, 1978). The interaction of starch and water does occur before the initial temperature of gelatinization is reached, as detected by the disappearance of polarizing crosses under a microscope. Before the onset of gelatinization, water is slowly and reversibly taken up. At this stage the mobility of water decreases as the temperature is increased from 20^{o}-60^{o}C and it is thought that water is being reversibly complexed with the starch molecules in the granule. Although this change is reversible at temperatures below the onset of gelatinization, continued exposure of the starch to water can cause changes in the granule itself (Gough and Pybus, 1971).

In a concentrated paste, the individual granules gelatinize and swell freely until all the available water has been imbibed. As they swell, the swollen starch granules become increasingly susceptible to shear disintegration. The binding forces in the granule also become weaker as heating is continued and the susceptibility of the granule to mechanical and thermal breakdown increases. When the granules have swollen to occupy the entire volume, some of the soluble components in starch, which had earlier diffused into the surrounding aqueous region, may now diffuse back into the highly swollen granules (Olkku and Rha, 1978). The system becomes a gel like mass held together by associative bondings. The hot starch pastes may be viewed as a mixture of swollen starch granules and granule fragments, together with colloidally and molecularly dispersed starch molecules.

The factors involved in the formation and determining characteristics of starch gels are: the type and size of starch granules, as well as their age and previous treatment; the paste concentration; cooking time and temperature; agitation during cooking; time and temperature of storage after cooking and types and amounts of added ingredients (Ott and Hester, 1965).

Several methods have been used to study starch gelatinization. Kofler hot state microscopy has been used by the method as described by Watson (1964). Other microscopic measurements have been reported by Seidemann (1963) and by Kainuma *et al.* (1968), who used photopastegraphs. Miller *et al.* (1973) also used photmicrographs, which are photographs, taken through a microscope. Miller *et al.* (1973) studied freeze-dried amylograph samples using scanning electron microscopy. Although SEM can demonstrate in detail the arrangement of structure in the various stages of gelatinization, the preparation of the sample for the microscopy may alter it so that it is no longer representative of the actual starch gel. Goto and Yokoo (1969) studied 40%, 45.5%, 47.6% and 50% starch suspensions. Higher concentrations lowered the gelatinization temperature and led to a higher peak consistency. Additives like protein and lipid are known to interfere in gelatinization of starch molecules.

2.2.1.1. Effect of protein on starch gelatinization

The gelatinization is more complex when starch in wheat flour is present with the natural protein. Protein and starch form complexes in the flour while being gelatinized. According to Takeuchi (1969) the starch-protein interaction is due to the attraction of opposite charges. Dahle (1971) has studied the starch binding effects of wheat flour protein and found that association of gelatinized wheat starch and wheat protein occurs at acid and neutral pH but is diminished at alkaline pH. Modification of the wheat protein by heat denaturation results in loss of the starch binding properties of the wheat protein. The consistency of the wheat protein-starch systems is altered by heat denaturation of the protein. It appears that protein forms complexes with starch molecules on the granule surface, preventing escape of exudates from the granule and therefore interfering with the increase in consistency.

From the theory of oppositely charged colloids it can be reasoned that at alkaline pH both starch and protein bear negative charges and complexing does not occur. At acidic pH wheat protein bears a negative charge and complexes can be formed. Berry and White (1966) have shown that the gluten of wheat flour increases the gelatinization temperature of wheat starch. Proteins and lipids present in flour also complex with each other and starch, thus complicating the overall picture (Olcott and Mecham, 1947). Naguchi *et al.* (1981) reported that the extrudates become denser and rigid when soy protein isolate was added to rice flour. It may be due to the fact that protein interfered with the gelatinization of starch by competing for available water absorption.

2.2.1.2. Effect of salt on starch gelatinization

Ganz (1965) suggests that salts may be used to control swelling of starch. When 2.5% NaCl was added to wheat starch suspensions, which were subsequently heated, peak consistency was markedly increased. This increase was associated with enhancement of ‘granule integrity’, i.e. the granule remains intact or experiences greater swelling before fragmentation occurs. Ganz (1965) associates the effect of salt with the presence of crystalline regions in the starch granule, which have binding forces of varying strengths or different accessibilities. At a temperature range of 60^{o}C to 70^{o}C, NaCl seemed primarily to affect the weak forces or readily accessible regions involved in swelling. Ganz (1965) assumed that NaCl inhibits the opening of these regions. NaCl also appears to have the secondary effect of either delaying swelling at higher temperatures or altering the course of fragmentation.

2.2.1.3. Effect of lipid on starch gelatinization

The polar lipids form a complex with the amylose, or linear starch fraction (Leach, 1965). Wheat starches contain about 0.6% to 0.8% lipid materials, according to Medcalf *et al.* (1968). These lipid materials are distributed throughout the granule. Addition of lipid in extrusion is generally found to retard the degree of gelatinization and affect dough rheology in the barrel (Madeleine, 1979; Schweizer *et al.*, 1986). Lipid also might function as insulating agent-preventing water from being absorbed by the starch granules (Hu, 1992; Marshall *et al.*, 1990). Lin *et al.* (1997) showed that the fat content had the most prominent influence on starch gelatinization. Increasing the fat content significantly decreased the degree of starch gelatinization during extrusion process. A higher fat content is expected to have a stronger insulating effect and further depresses starch gelatinization (Lin *et al.*, 1997).

2.2.2. Analytical techniques for monitoring gelatinization of starches

The molecular properties of starch molecule and the process of gelatinization can be monitored by various analytical tools like rheology, thermal analysis, FT-Raman spectra and microscopy.

2.2.2.1. Rheology of Starch

Rheology is the study of the deformation and flow of matter (Rao, 1999). Starch granules swell when heated in excess of water, and their volume fraction and morphology play an important role in rheological behavior of the starch dispersions (Bagley and Christianson, 1982; Da Silva *et al.*, 1997; Evans and Haisman, 1979). Starch and protein play major role in providing the desirable characteristics of food products from cereals, meat and other sources. Hence, from the practical point of view, the rheological characterization of foods and their constituents are very important, particularly in relation to structure, stability and process design. Despite the commercial significance of starch pastes and food systems containing starch, there is relatively limited fundamental information available on the rheological properties of starch based composite foods (Yang *et al.*, 2004). Rheological properties of individual components, such as wheat starch and whey protein were studied (Aguilera, 1995; Mangino, 1992; Wong and Lelievre, 1981), however the data on basic rheological properties (complex modulus, G*, storage modulus, G' and phase angle, δ) is lacking. Rheological properties of starch and cereal or non-cereal derived proteins have been reported over the years (Aguilera and Rojas, 1996; Chedid and Kokini, 1992; Muhrbeck and Eliasson, 1991). Yang *et al.* (2004) studied the basic rheological properties of food model systems consisting of starch-water, starch-protein, starch-sugar and starch-sugar-protein mixtures.

Starch and water dispersions heated above their gelatinization temperature behaved as viscoelastic pastes. Upon cooling, the paste thickens and may form an elastic gels if the dispersion has sufficient concentration (Ring, 1985). Starch gels are commonly regarded as composite systems, consisting of swollen particles embedded in a three-dimentional network of aggregated amylose chains. During cooling, the effect of hydrogen bonds in the gel matrix gradually increased, especially at low temperature, leading to increase in G' value. When the temperature decreased to 50^{o}C, G' reached its peak and then started to decrease, probably due to the contraction of gel volume during cooling (Tsai *et al.*, 1997).

In studying the dynamic rheology of starch from 45°-90°C, the initial increase in G’, could be attributed to the degree of granular swelling to fill the entire available volume of the system (Eliasson, 1986; Keetels and Van Vliet, 1994) and intergranule contact might form a network of swollen granules (Evans and Haisman, 1979; Wong and Lelievre, 1981). The lowering of G’ with further increase in temperature could be attributed to the melting of remaining crystalline structure, which resulted in swollen granules to become softer in texture (Tsai *et al.*, 1997). The extent of breakdown in G’ is a degree of disintegration of starch granules (Singh *et al.*, 2002). The differences in G’, G’’ and tan δ during the heating cycle may be attributed to the difference in the starch granule structure which in turn depends on their biological origin (Svegmark and Hermansson, 1993). Therefore the rheological properties of starch depended mainly on the interaction among close-packed granules and their rigidity during the heating process. Lii *et al.* (1996) reported the rheological behavior of gelatinized starch suspension was primarily due to intergranular interaction, such as entanglement between surface molecules of adjacent granules and the properties of the granules themselves.

Gelatinized starch dispersions are non-Newtonian fluids that may also exhibit yield stress at low shear rates (Evans and Haisman, 1979). It has been shown that dispersions of starches exhibit pseudoplastic flow behavior (Evans and Haisman, 1979; Rao *et al.*, 1997). Since granules in gelatinized starch dispersions have a size distribution, the rheological behavior of the dispersions depends on their size, size distribution and shape as well as interaction between the granules, the continuous phase viscosity and the rate and time of deformation (Barnes, 1989). Sasaki *et al.* (2004) have studied the effect of water-soluble and insoluble non-starch polysaccharides isolated from wheat flour on the rheological properties of wheat starch gel. Addition of water insoluble polysaccharide (WIP) increased the elastic and viscous component of starch gels, implying that WIP has great capability to hold water and increases the starch concentration in the continuous phase, resulting in increased starch molecule reassociation.

2.2.2.2. Differential scanning calorimetry

A combination of analytical techniques is used in order to examine the molecular organization and molecular dynamics in starch based materials at various molecular levels. Gelatinization degree can be investigated by means of various techniques. Differential Scanning Calorimetry (DSC) is one of the most commonly employed tools to study the thermal properties of starch molecule including gelatinization process (Biliaderis *et al.*, 1980, 1986; Ojeda *et al.*, 2000). In DSC analysis, samples are heated at a constant rate and the rate of absorption of heat is measured. The calorimeter consists of a sample and a reference container, both heated separately over a particular temperature range, and being kept at equal temperature. Variations in the heat capacity, enthalpy, melting and glass transition of the sample are detected by a difference in heat flow dQ/dt between reference and sample. Heat is absorbed during melting resulting in an endothermic peak. For the exact determination of the melting enthalpy, calibration with a sample of known melting heat like Iridium or Indium is necessary. Stevens and Elton (1971) first reported the application of DSC to measure the heat of gelatinization of starch. They observed a clear endothermic peak in the temperature region between 54^o and 73^oC for different starches and this was defined as the gelatinization temperature. Gelatinization properties of rice starch were studied using differential scanning calorimetry as a function of different water concentrations (Spigno and Faveri, 2004) and found that water content influence both gelatinization enthalpy and activation energy.

DSC monitors the changes in the physical and chemical properties of starches, offering a thermodynamic approach to the study of starch gelatinization (Donovan *et al.*, 1983). Liu *et al.* (1991) quantitatively correlated crystallinity loss with thermal events as measured by DSC. Melting of native starches and melting of recrystallised (aged) starch can be investigated to examine the extent of recrystallisation, recrystallisation kinetics and the relationship with starch structure, such as the amylopectin chain lengths (McIver *et al.*, 1968; Liu and Thompson, 1998; Lai *et al.*, 2000; Karim *et al.*, 2000). Differential scanning calorimetry is a valuable tool for obtaining information about starch - lipid interactions. The transitions of the amylose - lipid complex and the influence of lipids on starch gelatinization and retrogradation have been studied (Eliasson, 1994). Food processing like extrusion might lead to very different types of complex food matrices. Chemical modification of the starch also affects the thermal properties of the amylose - lipid complex. They observed a decrease in gelatinization enthalpy for a waxy maize starch in the presence of lipids, and the reduced retrogradation of waxy maize starch in the presence of lipids.

Carbohydrate-protein mixtures are commonly used for the production of snack foods by extrusion. Starch is biosynthesized as water insoluble granule in which crystalline regions are interspersed amongst continuous amorphous matrix (French, 1984).

At a sufficiently low temperature or limited content of plasticizer, a molecular motion becomes restricted as a glassy solid is formed. On heating or plasticizer addition, the mobility of amorphous polymer increases and the material become flexible or rubbery. Thus, the glass transition denotes the change from brittle to rubbery behaviour at a temperature T_g. The T_g depends on molecular characteristics, composition and compatibility of the components in the amorphous matrix (Kalichevsky and Blanshard, 1992; Roos and Karel, 1991). The most common method used to determine glass transition temperature (T_g) is Differential Scanning Calorimetry (DSC). For biopolymers the drop in storage modulus at T_g is much smaller than for synthetic polymers, due to polar and hydrogen bond interactions between macromolecules and presence of crystallinity or crosslinking (Moates *et al*., 2001; Kalichevsky *et al*., 1993). The majority of foods have a much more complex composition, with a protein-carbohydrate polymeric matrix that entraps low mass molecular weight components like fat, sugars and others. For such matrices, the interactions of proteins and carbohydrates with water, with the minor components, and with each other govern the structure-property relationships (Matveev *et al*., 2000). DSC can also be used to examine the influence of water and other plasticisers on the glass transition temperature of starches (Forssel *et al*., 1997; Kalichevsky *et al*., 1992).

Native and gelatinized rice starches were compared in their glass transition and enthalpy relaxation at various water contents using a differential scanning calorimetry (Chung *et al*., 2002). The contribution of structural aspects (absolute amylose, free amylose, lipid complexed amylose contents, amylopectin chain length distribution and relative crystallinity) to the gelatinization behaviour of rice starches has been studied using differential scanning calorimetry by Vandeputte *et al*. (2003). They classified the rice starches into waxy (T_p: 65.2-65.8^{o}C) and normal low T_p starches (62.8^o-67.0^{o}C), intermediate T_p starches (71.7-73.5^{o}C) and lower T_p starches, where T_p is the peak gelatinization temperature.

The addition of sugars and other polyols to starch-water systems elevates the gelatinization temperature. This elevation in gelatinization temperature will be greater when the concentration of aqueous solution is high and larger the molecular weight of added solute. Differential scanning calorimetric study showed that the onset of gelatinization is shifted to higher temperatures with the addition of sugars and glycerol (Perry and Donald, 2002).

Measurement of starch thermal transitions using differential scanning calorimetry has been carried out by Yu and Christie (2001). Multiple transitions and instability of water contained in starch make it difficult to study the thermal behaviour of starch materials using differential scanning calorimetry. Shogren (1992) studied the gelatinization of corn starch with 11-50% water and reported that the starch gelatinized at 190^{o}-200^{o}C in the range of water content of 11-30%. Only when the moisture content was above 30% did the amorphous region start to gelatinize at about 70^{o}C.

Any application of starch involves the gelatinization or melting of the granule structure. Therefore, much research has been done on the structural changes in starch granules induced by heating, as a function of moisture content using techniques such as differential scanning calorimetry (Gidley *et al.*, 1993). The glass transition temperature (T_g) of amorphous and native potato starch with 16% moisture was investigated using DSC (Thiewes and Steeneken, 1997).

In the thermal analysis of aqueous starch systems, four types of events may be encountered, depending on the moisture content: the so-called sub-T_g endotherm, the glass transition, various crystal melting processes and finally, the high temperature endotherm, which marks the transition into a thermoplastic melt (Tomka, 1991; Willenbucher *et al.*, 1995). From a practical point of view, the T_g marks the transition in mechanical properties between ductile and brittle.

Chung *et al*. (2004) characterized thermal transition of cross-linked corn starch using a differential scanning calorimeter in the presence of excess or limited water. When analysed with excess water, the glass transition temperature of the cross-linked starches was higher than those of native starches. However, in the DSC analysis with limited water, glass transition temperature (T_g) and heat capacity increment at T_g decreased by cross-linking.

Glass transition is defined as the transition due to the reversible change in an amorphous polymer between viscous or rubbery condition and a hard, relatively brittle one. Glass transition is a relatively new concept for the food industry. Low-moisture foods have a largely amorphous matrix that can undergo the so-called glass transition, which has a marked effect on physical properties. The glass transition is relevant to food processing operations such as freezing, drying and extrusion, and affects quality attributes such as texture, stability, flavour release and enzymic spoilage in low-moisture systems (Noel *et* *al.*, 1990). Moraru *et al.* (2002) illustrated the phase behaviour of a meat-starch extrudate. The degree of starch damage affected the ΔH significantly. Starch damage can occur by mechanical action during the milling processes and is known to give more water solubility and susceptibility to enzyme hydrolysis. A near linear relationship existed between the percentage of damaged starch in a sample and ΔH measured. When the sample was 100% damaged, ΔH was zero.

2.2.2.3. FT-Raman spectroscopy

Raman spectroscopy is primarily used in biochemistry to investigate protein structures and recently its use has become popular in determining the functions and interactions of proteins in a food matrix (Yu, 1977; Howell *et al.*, 2001; Li-Chan, 1996). Many studies using Raman spectroscopy have been reported on proteins, including protein-protein interactions, which occur during processing and storage of many food products (Howell, 1992). Raman spectroscopy can also provide accurate quantitative measurement of total degree of unsaturation of lipids, as indicated by changes in the ratios of C=C stretching band near 1660 cm^{-1} to the C=O stretching band near a wavenumber of 1750 cm^{-1} or CH_2 scissoring band near 1445 cm^{-1} (Jorabchi *et al.*, 1990; Jorabchi *et al.*, 1991). Raman spectroscopy offers advantages in comparison with other methods used to study vibrational spectroscopy and the most important one is the flexibility of sampling (Giles *et al.*, 1999). Solids can be analysed without any sample preparation. A number of organic compounds and functional groups can be identified by their unique pattern of absorption, and the intensity of the absorption may be used for the calculation of the relative concentration in the sampled entity (Wetzel and LeVine, 1999). The aromatic aminoacid phenylalanine showed a strong band at 1005 cm^{-1} for fish proteins. As the intensity of the band is not affected by the microenvironment or external factors the intensity of this band can be used as internal standard for normalization. Several Raman bands have been assigned as tyrosine residue vibration, which have an important role in the hydrogen bond formation. Among these bands the tyrosine doublet bands, which are located at the 850 and 830 cm^{-1} and the ratio of the peaks is useful for monitoring microenvironment around the tyrosine residue. If tyrosine residue is exposed, the ratio is high around 0.9-1.45; a low ratio indicates strong hydrogen bonding (Li-Chan *et al.*, 1994). The increase in tyrosine residues exposed on the protein surface, which can interact with water molecules as a hydrogen bond donor or acceptor (Li-Chan *et al.*, 1994; Parker, 1983;

Ogawa *et al.*, 1999; Badii and Howell, 2002). Studies on the Amide I and Amide III bands of Raman spectra showed that these regions are due to the secondary structure of polypeptide chains. The exact location of Amide bands depends on the hydrogen bonding and conformation of the protein structure (Frushour and Koenig, 1975). Amide I arises from C=O stretch and partly from N-H bending vibration. For proteins which contain a high level of α-helix the Amide I band is centered at 1645-1657 cm^{-1}, whereas, a higher content of β-sheet structure Amide I band is centered at 1665-1680 cm^{-1}, and proteins that contain a high proportion of undefined or random coil structure have Amide I band centered around 1660 cm^{-1}. In general, most proteins have mixed secondary structure, thus this area showed several components or shoulder (Li-Chan *et al.*, 1994)

The advent of high sensitivity, low noise detectors (such as Charge Coupled Devices [CCD]), and improvement in laser technology allows the use of Raman spectra to classify starches (Schuster *et al.*, 2000). Raman spectra of starch and gum have been studied (Vandenabeele *et al.*, 2000). Unlike the Raman spectra of the resins, there are no intense peaks in the 1800-1500 cm^{-1} regions and this differentiates the gums from proteinaceous and resinous media. These materials indeed do not contain aromatic or aliphatic C=C bonds. In contrast to the proteinaceous media, no amide I and III bands are present in this region. The rather broad peaks in the 1500-1200 cm^{-1} regions are ascribed here to CH deformations. The peaks at lower wave number are associated with CC and CO symmetrical stretching modes (1200-950 cm^{-1}) and COC sugar ring vibrations (950-800 cm^{-1}). The weaker peaks below 800 cm^{-1} are assigned to CC and CO deformations and skeletal breathing modes.

Complex study of the structure of practically important water soluble derivative of cellulose and starch with the use of couple calculations of conformations and vibrational spectra, FT-IR and Raman spectroscopy, mathematical treatment of spectral curves has been performed by Zhbankov *et al*. (2004). They found that characteristic of the Raman spectra of all polysaccharides with α (1→4) bonds between elementary links is the appearance of an intense band at 480 cm^{-1}. Raman spectroscopy has been used to identify modified starches with regard to their origin and type of modification (Dupuy and Laureyns, 2002). In general, the composite amide I band nominally designated as 1650 cm^{-1} was composed of a major peak at 1658-1660 cm^{-1} with shoulder on either side that were modeled as peaks at 1680 and 1630 cm^{-1} (Wetzel *et al.*, 2003). Experiments showed that Raman spectra could be applied to any mixtures of biopolymers, even when their spectra are highly overlapping. Pudney *et al.* (2003) measured concentrations of bulk phase separated systems (eg. Gelatin/dextran) using Raman spectroscopy.

Both Raman and FT-IR micro spectroscopy offer information on the molecular vibrations and structure of food samples. Raman spectroscopy has an advantage because of its ease of sampling, its higher resolution and the possibility for confocal measurements. However, the lower signal to noise ratio, the risk of damaging the sample with the laser, and especially auto-fluorescence of the sample may hamper its applicability and provide an option for use of FT-IR micro-spectroscopy (Thygesen *et al.*, 2003).

In order to determine the role of the skeletal base configuration of carbohydrate molecules, Zhbankov *et al.* (1997) made a comparative study using IR and Raman spectroscopy of theoretical calculations of the vibrational spectra of a series of carbohydrates differing in the configuration of CO (CH) bonds in various positions of the pyranose ring. They concluded that vibrations have a peculiar localization and that steric factors play an important role in the vibrational spectra of carbohydrates.

Infrared (IR) and Raman spectroscopy are complementary techniques that provide information on molecular structure. In Infrared spectroscopy different chemical bonds absorb at different infrared wavelengths depending on the atoms connected, the surrounding molecules and the type of vibration (for example stretching or bending). In Raman spectroscopy, the sample is radiated with monochromatic visible or near IR light from a laser (Thygesen *et al.*, 2003). This brings the vibrational energy levels in the molecule to a short lived, high energy collision state, which returns to a lower energy state by emission of a photon. Normally the photon has a lower frequency than the laser light (Stokes Raman scattering) and the difference in frequency of the laser and that of the scattered photon is called the Raman shift. The Raman shift corresponds to the frequency of the fundamental IR absorbance band of the bond. While IR spectroscopy detects vibrations during which the electrical dipole moment changes, Raman spectroscopy is based on the detection of vibrations during which the electrical polarisability changes (Pistorius, 1995). Measurements on samples that contain one or more of the fluorescent aminoacids (tyrosine, tryptophan and phenylalanine) or chlorophyll may prove difficult to study by Raman spectroscopy. However, the problem may be overcome if a Raman instrument is equipped with a near infrared laser instead of a laser in the visible range (Thygesen *et al.*, 2003).

On-line monitoring of starch gelatinization using FT-Raman spectroscopy was carried out (Schuster *et al.*, 2000). As soon as the gelatinization temperature was reached (58-62^{o}C) all bands decreased in intensity, only the bands at 1633 cm^{-1} and 3213 cm^{-1} increased. This can be attributed to the uptake of water into the crystalline structure of the starch, and following, the gel formation, which causes the break of intramolecular and intermolecular hydrogen bonds due to the uptake of water into the starch particles, and the formation of new hydrogen bonds with water (3213 cm^{-1}).

2.3. Extrusion Process

The term extrusion refers to “to shape by forcing through specially designed opening often after a previous heating of the material” (Webster’s dictionary definition). An extruder is a machine, which shapes materials by the process of extrusion. Extrusion cooking is a versatile and very efficient technology, widely used in food processing. There is a trend in the food industry to develop convenience products, such as puffed snack foods and breakfast cereals of high nutritional value. The main raw material used for food extrusion is starch in combination with other macro or micronutrients. Early studies on extrusion processing of starch are limited to investigating the effects of variables, such as temperature and feed moisture content, on product properties (Anderson *et al.*, 1969; Mercier and Feillet, 1975). To increase protein content and nutritive value, protein source such as fish can be included in snack formulations prior to extrusion. Research on extrusion processing of fish muscle started in the 1980’s (Choudhury and Gogoi, 1995). Fish flavor is desirable in snacks produced for the international market.

Ledward and Tester (1994) studied the molecular transformations of proteinaceous foods during extrusion processing. They highlighted the possible importance of electrostatic interactions in the extruder and the chemistry involved in the formation of covalent bonds that may be of importance in texture development. Normally, in extrusion cooking, a reduction in the size of the die aperture causes an increase in die resistance and, therefore, an increase in die pressure. However, with some experimental recipes based on rice flour, following a reduction in the diameter of twin circular dies from 4 to 3 mm, the reverse was found to be true under certain conditions of screw speed, using an APV Baker twin-screw extruder (Janes and Guy, 1995). The swelling of starch dough during extrusion as a function of die geometry, shear rate and dough rheology has been characterized by Robert *et al.*, (1993).

Extrusion cooking is used for the manufacture of food products such as ready-to-eat breakfast cereals, expanded snacks, pasta etc. The process is reviewed by Harper (1981), Linko *et al.* (1981) and Mercier *et al.* (1989). The first food extrusions involved the use of piston or ram type extruders to stuff casings in the manufacture of sausages and processed meats (Harper, 1981). The development of food mincer may have been the first use of a single screw food extruder. The initial application of the single screw extruder, which revolutionized an entire industry, was its use as a continuous pasta press in the mid 1930s. Here low shear extrusion screws characterized by deep screw flights, cause little heat or precooking to occur in the dough.

General Mills, Inc. was the first to use an extruder in the manufacture of ready-to-eat (RTE) cereals in the late 1930s. Expanded corn collets or curls were first extruded in 1936 but the product was not commercially developed until 1946 by the Adams Corp. Collet extruders are characterized by an extremely high shear rate within the flights of the screw and grooves in the barrel. No external heat supplied, such extruders are called autogenous. Initial studies on extrusion cooking of corn were done by Conway *et al.* (1968); Anderson *et al*. (1969) and Conway (1971 a, 1971 b). The authors felt that low-moisture extrusion, which provided high temperatures and shear rates, enhanced degradation of starch and the formation of dextrins.

The desire to precook animal feeds to improve digestibility and palatability led to the development of the cooking extruder in the later 1940s and has greatly expanded the application of extruders in the food industry (Harper, 1981). The wide range of moisture contents (10 to 40%), feed ingredients, cooking temperatures (110^o to 200^oC) and residence times greatly enhances the versatility of the modern day food extruder. In 1960s RTE breakfast cereals were developed which were cooked and formed continuously with a one step process on a cooking extruder. Pet foods are the largest single product type produced on extrusion equipment.

Recently twin-screw extruders have been used to process food products (Janes and Guy, 1995; Choudhury and Gautam, 1999). Two types of twin screw extruders are employed, one where the two screws rotate the same direction and the other, where the screws rotate in opposite directions. These provide better control of residence time and internal shear of the food ingredients for products that are very heat sensitive and can operate at very low product moistures. Twin-screw extruders are used for production of a wide variety of food products and extrudate characteristics from starchy and proteinaceous ingredients depend on physicochemical changes occurring during extrusion (Choudhury and Gautam, 1999). Twin-screw extruders are operational at very low feed moisture (6%) requiring no or minimum post-extrusion drying (Harper, 1989; Dziezak, 1989). An increasingly important role for food extruders appears assured in the food processing industries because of an expanding interest and demand for fabricated foods (Smith, 1975).

These trends are being driven by an increasing world population and a shrinking conventional energy supply. Both focus on the need to transform raw agricultural products consisting of starch, plant protein and fat directly and efficiently into food having high acceptability. The ability of the extruder to combine cook and texturize food components quickly, continuously and efficiently makes it ideally suited for this task (Camire *et al*., 1990). Because of the complexity and variability of food ingredients, reducing food extrusion to a simple mathematical equation is highly unlikely in the foreseeable future. However, the application of basic engineering and scientific principles, as far as possible, can lead to an increased understanding of the complex process and greatly enhance the effectiveness of the technologist in applying the food processing tool called extrusion (Harper, 1981).

The extensive theoretical developments made in the area of plastics extrusion (Tadmor and Klein, 1978; Tadmor and Gogos, 1979; Fenner, 1980; Janssen, 1988; Gopalakrishna *et al*., 1992) are an excellent place to begin the development of a model of a food extruder. Unlike plastic where only a single type of plastic is extruded, food extrusion uses an infinite variety of food ingredients as feed materials. Often the ingredients are biopolymers of starch or protein, but their exact character depends upon their source, age, prior treatment etc. (Harper, 1981). In food extrusion the ingredients and water react in a complex cooking process, which causes extensive alterations in the chemical and physical nature of the extruded materials (Bryant *et al*., 2001).

In the feed section of the extruder, relatively free-flowing granular particles of the food ingredients exist. This granular material is caught between the flights of the screw channel and is conveyed in a manner similar to the action of a screw conveyor. Little or no internal shear of the food takes place in the solids conveying section as contrasted to shear flow in the metering section (Harper, 1981). Transition section of a food extrusion screw is where the food ingredients change from a raw or uncooked granular material to the plastic like dough, which exists in the metering section of the screw. In the food system, the change of physical character is associated with a set of chemical reactions like hydration and denaturation of protein, hydration, gelatinization and dextrinization of starch, browning where free ε-amino groups of the protein react with reducing sugars, destruction of antigrowth factors, vitamins and enzyme systems which occur in raw foods and finally destruction of microorganisms which exist in the raw food (Thakur and Saxena, 2000; Chuang and Yeh, 2004; Harper, 1981). The extruder die is the shaped hole through which the food dough flows at the extruder exit. The cross sectional area of the die gives the shape to the extruded food product.

The principal advantages of the modern food extruder, leading to their expanded role in the food processing industry, were given by Smith (1969), Bjorck and Asp (1983), Mustakas *et al.* (1964), Harper and Jansen (1985), Cheftel (1986) and Faraj *et al.* (2004).

1. Versatility - a wide variety of foods can be produced on the same basic extrusion system using numerous ingredients and processing conditions.
2. High productivity - an extruder provides a continuous processing system having greater production capability than other cooking/forming systems.
3. Low cost - labor and floor space requirements per unit of production are smaller than for other cooking/forming systems enhancing cost effectiveness.
4. Products shapes - extruders can produce shapes not easily formed using other production methods.
5. High product quality - the HTST heating process minimizes degradation of food nutrients by heat while improving digestibility by gelatinizing starch and denaturing protein. The short, high temperature treatment also destroys most undesirable factors in food. Some of these heat denaturable factors are antinutritional compounds such as trypsin inhibitors, hemagglutinins, and gossypol and undesirable enzymes such as lipases or lipoxidases, microorganisms and other food borne pests.
6. Energy efficient - extrusion processing systems operate at relatively low moisture while cooking the food products. Lower moisture reduces the quantity of heat required for cooking and redrying the product after cooking.
7. Production of new foods - extruders can modify vegetable proteins, starches, and other food materials to produce a variety of new food products.

No effluents, that is lack of process effluents is an important advantage since stringent controls are being placed upon food processors to prevent their releasing pollutants to the environment.

2.3.1. Cooking extruders

Several designs are possible for extruders. Important among them is screw extruders consisting of flighted screw(s) rotating within a sleeve or barrel (Harper, 1981). The action of the flights on the screw pushes the plasticized material forward and creates the pressure behind the discharge die so that it extrudes through the opening.

Cooking extrusion combines the heating of food products with the act of extrusion to create a cooked and shaped food product. Cooking extrusion can be described as a process whereby moistened, starchy, and/or proteinaceous foods are cooked and worked into a viscous, plastic-like dough (Chiruvella *et al*., 1996). Cooking is accomplished through the application of heat, either directly by steam injection or indirectly through jackets, and by dissipation of the mechanical energy through shearing occurring within the dough.

The results of cooking the food ingredients during the extrusion process are the gelatinization of starch, the denaturation of protein, the inactivation of many raw food enzymes which can cause food deterioration during storage, the destruction of naturally occurring toxic substances such as trypsin inhibitors in soybeans, and the diminishing of microbial counts in the final product (Chuang and Yeh, 2004; Thakur and Saxena, 2000; Alonso *et al*., 1998; Camire *et al*., 1990; Wiriyaumpaiwong *et al*., 2004).

The temperatures reached by the food during cooking extrusion can be quite high (200^{o}C) but the residence time at these elevated temperatures is very short (5 to 10 sec) (Harper, 1981). For this reason, extrusion processes are often called HTST (high-temperature/short time) process. They tend to maximize the beneficial effects of heating foods like improved digestibility, while minimizing the detrimental effects like browning, inactivation of vitamins and essential amino acids, production of off-flavours, etc. (Camire *et al*., 1990). Once cooked the product is forced through a die at the extruder discharge where it expands rapidly with some loss in moisture because of a rapid decrease in pressure. After expansion and cooling/drying, the extrudate develops a rigid structure and maintains a porous texture.

2.3.2. Extrusion process variables

There are number of independent variables in food extrusion process like feed ingredients, moisture, screw and barrel design (Chuang and Yeh, 2004), die design, screw speed, jacket temperatures, pre-conditioning and feed rate (Bhattacharya and Hanna, 1987; Bhattacharya and Hanna, 1988; Cervone and Harper, 1978; Harper, 1981). Both temperature and water are the key elements affecting the internal structure as well as the viscoelastic properties of extruded cereals (Brent *et al*., 1997). The ultimate character of the food extrudate is dependent upon the composition of the feed ingredients (Anderson *et al.*, 1969; Mercier and Feillet, 1975; Peri *et al.*, 1983; Mercier *et al.*, 1980; Kim and Rottier, 1980). It is therefore, critical that the feed ingredients be carefully specified and controlled. But it is difficult, because food materials are biological and can have natural variability. The dependent variables in food extrusion are viscosity of the material (Guha *et al.*, 1998), shear rate occurring within the screw channel, pressure at the discharge, residence time (Chuang and Yeh, 2004) and product characteristics. Typical product characteristics are density, texture, strength, gelatinization, fiber formation, water uptake, flavor, color and appearance.

The effect of the extrusion process variables, such as length/diameter (L/D) ratio of the extruder, feeding rate, temperature of extrusion and screw speed on the rheological behaviour of blends of partially dried low-cost minced fish and wheat flour were studied (Bhattacharya *et al.*, 1992). The L/D ratio of the extruder affects the rheological behaviour of the food dough during extrusion and hence, the L/D ratio should also be considered as a process variable. Mixing is important within the food extruder to assure uniformity of the extrudates (Seker *et al.*, 2003). Mixing occurs within an extruder due to the existence of laminar shear flow. The effects of location and spacing of kneading elements on specific mechanical energy input and product attributes during twin-screw extrusion of pink salmon (*Oncorhynchus gorbuscha*) muscle and rice flour blends were investigated (Choudhury *et al.*, 1998). Incorporation of kneading elements increased specific mechanical energy, expansion ratio and water solubility index, but decreased bulk density and Warner-Bratzler shear stress.

The effect of die resistance and minor ingredients in a food extrusion cooker during extrusion of rice flour has been studied (Janes and Guy, 1995). The general consequences of changing die diameters were considered (Guy and Horne, 1988). When the die diameter was decreased from 4 mm to 3 mm there was increase in specific mechanical energy input, greater expansion of the product and greater increase in specific volume of the product. The morphological changes to the final products were complicated because of different rates of change of expansion in different planes with feed moisture content, screw speed and die diameter (Guy and Horne, 1988). The higher die pressure coincides with low kneading zone pressure and a temperature drop after the end of the kneading zone (Janes and Guy, 1995).

Gomez and Aguilera (1983) studied the effect of extrusion on ground corn at moisture contents from 23.7-7.6%. Decreasing moisture content resulted in increase in water solubility index, enzyme susceptibility, and degree of gelatinization and blue values, while water absorption index and water insoluble carbohydrates decreased. The moisture content of the feed ingredients is one of the principal techniques controlling the extrusion process (temperature and rate) and extruded product characteristics (Brent *et al.*, 1997). Specifically, the moisture content of the feed will affect product density, expansion cooking, product rehydration, starch gelatinization etc. The moisture can be added to ingredients in many ways including water, steam and blends of ingredients such as emulsions and syrups. The hardness of the water used in extrusion also affects the quality of the extruded product (Maga and Desrosiers, 1979).

2.3.3. Types of extruders

The primary types of extruders are single- and twin-screw extruders. A single-screw extruder is considered as a friction pump and a twin-screw extruder as a positive displacement pump (Seker *et al.*, 2003). The initial development of food extrusion process was developed with single screw extruder. At a later stage development of twin-screw extruder became very popular commercially because of flexibility in the process parameters. In the case of a twin-screw extruder with closely intermeshing screws, the food is transported in so called C- shaped chambers, which are enclosed by the neighbouring intermeshing screw and the barrel wall (Van Zuilichem *et al.*, 1999). The food powder is taken up at the feed port in the C-shaped chamber of one screw, is conveyed, internally mixed, forced through the gaps and clearances of this screw or eventually transferred to the neighbouring screw and finally this C-shaped chamber delivers its content at the die (Janssen, 1978; Van Zuilichem *et al.*, 1983). Twin-screw extruders are operational at very low feed moisture (6%) requiring no or minimum post-extrusion drying (Harper, 1989;

Dziezak, 1989). In addition, they enable greater flexibility of operations to control product attributes by monitoring a desired time, temperature and shear history because of the additional independent variable screw configuration (Choudhury and Gautam, 1999).

Effects of barrel temperature (81°-149°C) and screw speed (315-486 RPM) on extrusion processing of sago starch in a co-rotating twin-screw extruder under a high moisture system (34-47%) was investigated using response surface methodology (Govindaswamy *et al.*, 1996). They observed that thermo mechanical processing of sago starch in the twin screw extruder at the high moisture system led to shear induced limited degradation and starch phase transitions. They also showed that gelatinization was the fundamental mechanism in this high moisture system rather than dextrinization. The case of breakfast cereal production was challenging, because of the combination of a wanted certain viscosity (spoon behaviour), which can only be achieved with low shear equipment like a counter-rotating twin screw extruder (Van Zuilichem *et al.*, 1999). Mixing effects of constituting elements of mixing screws in single and twin-screw extruders have been investigated (Van Zuilichem *et al.*, 1999).

2.3.4. Residence time

There are several reactions, such as gelatinization of starch, denaturation of protein, enzyme inactivation and Maillard reactions occur in the extruder. The average extent and uniformity of the reactions are affected by the residence time distribution (RTD) (Chuang and Yeh, 2004). The RTD is a plot of residence time against the fraction of flow having this residence time. The measurement and reporting of residence time distribution in food extrusion is very important. Van Zuilichem *et al.* (1973) reported measuring RTDs in corn grits extrusion using a radio-tracer technique with ^{64}Cu. Copper was chosen as a tracer because it has a half life of 12.7 hr. Residence time data were obtained by Bigg and Middleman (1974) using a red food color dissolved in the liquids being extruded. Van Zuilichem *et al.* (1983) were the first to measure RTDs in the extrusion of biopolymers. The studies on RTD of food materials in an extruder are to understand the flow patterns and are enabling one to predict the performance of an extruder.

The effect of three types of screw elements, forward, mixing disc and pin-mixing element on residence time distributions of glutinous rice flour in a single screw extruder with different die opening areas have been investigated (Chuang and Yeh, 2004). Both mixing disc and pin mixing element yield longer residence time, higher specific mechanical energy and higher extrudate temperature compared with those for the forward screw element. Seker *et al.* (2003) studied the effect of a mixing element in single and twin-screw extruders on the amount of phosphorus incorporated into starch for the chemical modification of starch that has applications in the food and paper industry. They observed that increased screw speed reduced the residence time in both single- and twin-screw extruders with and without a mixing element. Screw speed of 220 rpm in a single screw extruder with a mixing element, 180 rpm in a single screw extruder without a mixing element, and 160 rpm in a twin screw extruder with and without a mixing element resulted in similar residence time.

2.3.5. Extrusion of starches and starchy materials

A very high fraction of all human food energy is in the form of cereals and/or starchy foods. Extrusion cooking which is a continuous high-temperature, short time process has become a popular economical process to formulate new cereal foods (Camire *et al.*, 1990). In the developing world, where food is in short supply and commands a higher percentage of people's expendable income, cereals constitute the major proportion of dietary energy. In addition to food energy provided by starch, it provides much of the texture, mouth feel and structure of the foods that are consumed. The transformation of starchy materials to materials having a crispy expanded texture, which can readily take on water, requires cooking or gelatinization. There are ample reports in the literature concerned with the functional properties of extruded starches (Lawton *et al.*, 1972; Mercier and Feillet, 1975; Linko *et al.*, 1981; Gomez and Aguilera, 1984). The principal constituent of all cereals and starchy root crops is the biopolymer called starch. The ratio of amylose to amylopectin in starch influences the textural properties of the finished extruded product as described by Murray *et al.* (1968). Amylopectin promotes puffing, giving a very light and fragile product. Conversely, starches, which are high in amylose or from root origins, tend to produce harder products with limited puffing. For puffed snacks, 20% amylose in the starch is considered minimum while amylose contents greater than 50% give an exceedingly dense product lacking puffiness. When extruded products containing 10 to 15% moisture are fried, the amount of oil retained is related to the amount of puffiness.

Extruded foods and cereals, which are primarily starch, represent an important and expanding food area. The important functions of extruder in this processing are pre cooking and gelatinization of the starch, providing a desired shape to the product, and giving the food expanded, crisp and pleasant characteristics. Sanderude (1969a, 1969b) and Wells (1976) have described a variety of dry milled cereal products that are used in the production of extruded foods. Rice, a cereal grain which is low in protein and fat, light color and ability to expand makes it ideal for cereal and snack products. The relative expense of rice, compared to other cereal grains, is main drawback. Wheat contains the protein gluten, and requires high moistures and temperatures for expansion. The extruded products show the carbohydrate embedded in a matrix provided by the gluten. Soft spring wheat, with lower gluten levels, will yield a tender expanded product than with semolina or hard winter wheat.

The unique attributes such as bland taste, attractive white color, hypoallergenicity and ease of digestion make rice flour an attractive ingredient in the manufacture of new cereal foods (Kadan *et al.*, 2003). During extrusion, the starch undergoes profound physicochemical changes, such as gradual alterations in x-ray diffraction and differential scanning calorimetry patterns (Kadan and Pepperman, 2002). The physico-chemical changes, as a result of extrusion, result in new functional properties (Bryant *et al.*, 2001). The functional properties of long-grain and short-grain rice flour extruded at 70^{o} to 120^{o}C and 22% moisture are reported (Kadan *et al.*, 2003). The bulk density stayed unchanged, whereas, both water absorption and water solubility indices increased with an increase in extrusion temperature.

Kim and Rottier (1980) studied the modification of wheat semolina by extrusion. The wheat semolina was extruded at 60^{o}, 90^{o}, 125^{o}, 150^{o} and 190^{o}C. They found that when the extrusion temperature was increased to 125^{o}C, the amount of damaged starch increased very sharply. Bredie *et al.* (2002) studied the effect of temperature and pH on the generation of flavor volatiles in extrusion cooking of wheat flour. Analysis showed that sulphur and nitrogen-sulphur- containing heterocyclic compounds as possible contributors to the sulphury and rubbery odors observed in extrudates produced at the higher temperature and more alkaline conditions.

Extrusion behaviour or potato starch has been studied by Della valle *et al.* (1995). They found that potato starch extrusion exhibits two main differences when compared to other starches: high melt viscosity and early melting in the extruder. Both explain the difficulties usually encountered when processing this material, characterized by high-energy requirement. Early melting may be due to transition temperatures lower than the values encountered for cereal starches. High melting point is linked to the higher molecular weight of potato starch. The extrusion behavior of cereal starches has been thoroughly studied compared to potato starch (Colonna *et al.*, 1989).

2.3.6. Behaviour of starch during extrusion

Limited information is available on changes occurring in the starch fraction of foods during extrusion cooking although most physical and sensory properties of extruded products depend on the extent of degradation (Gomez and Aguilera, 1983). From the initial studies on extrusion cooking of corn, it has been observed that low moisture extrusion provided high temperatures and shear rates enhancing degradation of starch and formation of dextrins (Conway *et al,* 1968; Anderson *et al.*, 1969 and Conway 1971a, 1971b). Gomez and Aguilera (1983) studied the changes in the starch fraction during extrusion cooking of corn. Degradation of wheat starch during single screw extrusion has been studied (Diosady *et al.*, 1985). Both the studies concluded that dextrinization appears to become the predominant mechanism of starch degradation during low moisture, high shear extrusion.

The starch conversion process is studied in the rheological region of the extruder, which is often the last few turns of the screw, where the material is treated as a non-Newtonian fluid. Extrusion cooking for the manufacture of food products such as ready-to-eat breakfast cereals, expanded snacks etc. has been reviewed by Harper (1981), Linko *et al.* (1981) and Mercier *et al.* (1989). The material inside extruder gets compacted and then softens and gelatinizes and/or melts to form a plasticized material (dough), which flows downstream in the extruder channel. Simultaneously, the materials undergo chemical and physical transformations due to thermal and shear effects. Later the material is shaped by flow through the die at the end of the extruder. The process of extrusion cooking is not well understood because it involves both chemical and physical changes in the material and due to interdependence of product properties and quality. The chemical changes determine the extent of molecular modification such as starch gelatinization (Chiruvella *et al.*, 1996). The physical changes affect the textural properties such as hardness, elasticity and chewiness as well as other sensory attributes like color and flavour which are the primary factors in how a product is received by the consumer. During extrusion of food materials the heterogeneous nature of the material, the phase transitions, the denaturation of protein and other ingredients and the strong dependence of the properties on the moisture level and on temperature, complicate the determination of the physical and rheological properties of the food material undergoing thermal processing.

Waxy maize starch was modified by twin-screw extrusion cooking in the presence and absence of gelatin (Wulansari *et al.*, 1999). They observed that at constant specific mechanical energy and extruder heater temperatures, the degree of starch conversion decreased with increasing levels of gelatin.

2.3.7. Effect of sucrose on extrusion process

Sucrose is a common additive in commercially extruded foods, particularly breakfast cereals, and is incorporated into these products in concentrations upto 50% by weight (Hsieh *et al.*, 1990). Godshall (1988) reported that sugar contributes to binding, flavor and browning characteristics, which are critical for controlling the texture and mouth-feel, and acts as a carrier and potentiator of other flavor components. In the presence of adequate moisture during extrusion (above 16% weight), the effect of sucrose on extrudates structure manifests itself as an increase in product density and a reduction in expansion with increasing sucrose concentration (Moore *et al.*, 1990; Ryu *et al.*, 1993; Jin *et al.*, 1994). The effect of sucrose on the structure, mechanical strength and thermal properties of corn extrudates has been studied by Barrett *et al.* (1995). Sucrose has pronounced effects on the processability and structure of corn extrudates. Sucrose induces reduction in extrusion specific mechanical energy (SME) and in product expansion probably reflects the reduction in melt viscosity caused by replacement of starch-based material with sucrose. Such an effect would reduce shear conditions in the extruder and inhibit product expansion. These consequences are more pronounced in high moisture content formulations in which melt viscosity is relatively low, even prior to addition of sucrose. The structural changes in extrudates with the addition of sucrose have been attributed to competition for moisture (Hsieh *et al.*, 1990) and inhibition of gelatinization (Sopade and Le Grys, 1991) during expansion. High level of moisture prevents viscous dissipation of energy and product expansion, but facilitates operations such as emulsification, protein gelation, restructuring and shaping of comminuted meat and fish, and micro coagulation and/or fibrillation of specific protein constituents (Cheftel *et al.*, 1992).

2.3.8. Effect of lipid on extruded products

The presence of lipids in the ingredients used for extrusion or the added lipids during extrusion will have significant bearing on the final quality of the extrudates. The increase in lipid oxidation was observed with the increase in extrusion temperature (Rao and Artz, 1989). Extruded foods, particularly expanded products are susceptible to lipid oxidation. Lipid oxidation is a major cause of food deterioration, but limited success has been achieved in preventing this group of reactions from occurring in extruded foods. Viscidi *et al*. (2004) studied the effect of complex phenolic compounds on lipid oxidation in extruded oat cereals. They concluded that addition of antioxidants to foods prior to extrusion could result in more stable products.

2.3.9. Extrusion with fish mince

Research on extrusion processing of fish muscle started in the 1980's (Choudhury and Gogoi, 1995). The aim was to use underutilized species, by-catch fish, and muscle recovered from by-products of filleting, canning and surimi operations. Literature on low-moisture extrusion of fish muscle is rather scanty (Choudhury and Gogoi, 1995). Some studies have been reported to develop dry expanded snack food products from low-moisture blends of fish muscle (mince fish from different species) and starchy ingredients using single and twin-screw extruders (Yu *et al*., 1981; Kristensen *et al.*, 1984; Maga and Reddy, 1985; Venugopal, 1987; Clayton and Miscourides, 1992; Choudhury, 1995; Gogoi *et al.*, 1996a, 1996b; Gautam *et al*., 1997; Choudhury *et al.*, 1998). Generally, incorporation of fish proteins reduced extrudate expansion and increased hardness. Studies have shown that the undesirable effect of fish proteins on extrudate expansion and hardness can be reduced by the incorporation of mixing elements in the screw profile (Gogoi *et al*., 1996a, 1996b; Gautam *et al*., 1997; Choudhury *et al.*, 1997; Choudhury *et al.*, 1998).

Suja and Basu (1998) developed fish mince based extruded products using a twin-screw extruder. The problem of product surface cracking could be overcome by optimizing moisture in the ingredients and the temperature of extrusion. Shah *et al.* (1999) produced fish crackers (keropok) by extrusion with addition of whey protein concentrate. Whey protein concentrate (WPC), at 3, 9 and 15%, was added to a fish cracker formulation consisting of tapioca starch, minced fish and salt. The mixture was extruded and the extrudate cut, dried and fried. Expansion decreased with increasing WPC whereas, bulk density increased. Colour darkened with addition of WPC. Fish crackers with 9% WPC found better acceptance by consumers.

Co-extrudates were produced from mixtures of soy and fish proteins by thermal extrusion (Murray *et al*., 1980). It was found that the texture of the co-extrudates depended strongly upon both the ratio of protein to water and vegetable to fish protein. Texture was improved by the addition of fish, which also reduced the temperature required for optimal texturization. Addition of fish also raised the level of essential amino acids.

2.3.10. Texturization

Thermoplastic extrusion is widely used for the texturization of proteins, polysaccharides and their blends (Smith, 1976). It is mainly in the field of texturization that extrusion cooking at high moisture levels represents a novel and useful technique, clearly different from other continuous protein texturization processes (Lillford, 1986). Isobe and Noguchi (1987, 1989) and Gwiazda *et al.* (1987) were the first to prepare really fibrous gelled structures by extrusion cooking of defatted soy flour at moisture content of 60%. Frazier *et al.* (1983) optimized process variables in extrusion texturing of soya. Extrusion cooking is capable of converting soluble, globular legume proteins into material with the fibrous and chewy texture of meat. Extrudate evaluation using Warner-Bratzler shear force showed feed moisture and protein has the greatest effect on texture, while temperature was the next most important factor. Although extrusion cooking has been applied with great commercial success to the production of shaped pasta products and ready-to-eat breakfast cereals for over 50 years, its application to the texturization of vegetable proteins is a more recent innovation. However, although the last 20 years or so have seen a great deal of activity in this area and textured products from protein-rich sources, especially soy flour is now readily available commercially.

Extrusion process can be applied for the development of high value surimi based seafood products in which the surimi paste is extruded into various shapes that resemble shellfish such as shrimp, lobster, crab and scallop and thereby increase the value of by-catch which are normally considered as under-utilized for human consumption (Jeyasekaran and Shetty, 1992). Texturization has been carried out in mince from sardine, squid or octopus. In all cases, the gelled band displayed a multiplayer structure, with the layers being aligned in the direction of extrusion flow. The process has been patented (Sasamoto *et al*., 1987).

Miyano *et al*. (1992) studied the change in myofibrillar protein of fibrous product from walleye pollack (*Theragra chalcogramma*) surimi by extrusion cooking. Frozen surimi of walleye pollack was ground with 2.5% NaCl and cooked by a twin-screw extruder at 160^o-170^oC of barrel temperature. The extruded product thus prepared was solubilized and subjected to analysis of the subunit composition of myofibrillar protein by SDS-polyacrylamide gel electrophoresis. The results revealed that not only myosin heavy chain but also other components, such as actin, tropomyosin, troponin and myosin light chains were all involved to form a high molecular weight product during extrusion cooking.

2.3.11. Extruded snack foods

Snack foods have become an integral part of the eating habits of the majority of the world’s population (Thakur and Saxena, 2000). Extrusion has been used to produce a wide variety of snack foods (Suknark *et al.*, 2001; Harper, 1981; Viscidi *et al*., 2004). Carbohydrate-protein mixtures are commonly used for the production of snack foods by extrusion (Moraru *et al*., 2002). Snack foods comprise a very large variety of items including potato chips, popcorn, crackers, nuts and extruded snacks. Extruded snacks come in a variety of shapes other than the common collets and curls including tubes, wheels, rings, hats, mushroom sticks and scoops (Smith, 1974, Liggett and Ziemba, 1969).

Production of acceptable fish crackers prepared by the extrusion method has been standardized (Yu, 1981). Crackers (in Malaysia known as 'keropok') are popular snack foods in Malaysia and other South East Asian countries. Traditionally 'keropok' is prepared by forming dough from a mixture of manioc flour, comminuted fish and water. Fish:flour ratios of 20:80, 30:70, 40:60, 50:50 and 60:40 were used. Die section was maintained at a temperature of less than 100^oC. The temperatures for the second stage were varied from 60^o to 140^oC. After extrusion the product was cut into pieces and then dried in a forced-air cabinet drier at 70^oC for 6 hours to give final moisture content of 8-9%. For the product extruded at temperature of 100^oC and above, panelists found no significant difference between the traditionally prepared control and the extruded samples.

2.3.12. Extruded product expansion

The raw material will be subjected to high shear and temperature inside the extruder. The pressure and the temperature at the die end will be enormous. When the material comes out of the die it will be exposed to low atmospheric pressure and temperature, which results in the flashing of water vapor, which in turn will result in product expansion. Previous studies have shown that when incorporating protein into an extruded snack food there is decrease in expansion and increase in hardness of the final product, both of which are undesirable (Choudhury and Gautam, 2003; Choudhury *et al*., 1998; Onwulata *et al.*, 1998; Onwulata *et al.*, 2001). Siaw *et al.* (1985) stated that in the extrusion cooking of wheat flour, the reduction of expansion is due to the presence of wheat protein and this effect may be independent on the type of the protein present.

The expansion ratio of extrudates based on potato starch and isolated soybean protein was measured for a range of compositions and die diameters (Yuryev *et al*., 1995). They found that the expansion ratio was influenced by the multiphase nature of the melt as well as its rheology. By changing the cooling regime of the biopolymer melt at the die outlet, expanded or non-expanded extrudates can be prepared (Guy and Horne, 1988). Chinnaswamy and Hanna (1990) studied the relationship between viscosity and expansion properties of various extrusion cooked corn grain components. They found that the apparent viscosity of the extrusion cooked starch or flour affected expansion more than did the extrusion pressure. The quality attributes of ready-to-eat foods and cereals greatly depend on their crispness, which in turn depends on the expanded volume.

The extrusion technology replaced most of the conventional technologies to produce expanded cereals and other similar products. Chinnaswamy (1993) studied the expansion of cereal starches. All cereals and starches do not expand equally due to raw material quality differences. The branched structures of starches and their contents seem to control extrusion-expansion of cereals. It appears that proportion of branched fraction of starches affect expansion volume greatly.

2.3.13. Nutritional aspect of extruded products

The changes in the chemical and nutritional qualities of food during extrusion cooking have been reviewed (Bjorck and Asp, 1983; Cheftel, 1986; Camire *et al*., 1990). Cheftel (1986) reviewed the mechanisms underlying beneficial or detrimental changes in the bioavailability and in the content of nutrients that may take place during extrusion, as well as the influence of process conditions of food mix composition. Special emphasis is placed on the physico-chemical and chemical modifications of protein, starch and dietary fibre. Like other processes for heat treatment, food extrusion cooking may have both beneficial and undesirable effects on nutritional value. Beneficial effects include destruction of antinutritional factors and gelatinization of starch. On the other hand Maillard reactions between protein and sugars reduce the nutritional value of the protein. Heat-labile vitamins may be lost to varying extents (Bjorck and Asp, 1983).

The effect of extrusion cooking on the nutritional value, storage stability and sensory characteristics of an indigenous maize-based snack food were examined (Lasekan *et al.*, 1996). They observed a small loss (10%) of available lysine and an appreciable reduction in protein dispersibility index (PDI). In addition, storage at high temperature (40^{o}C) significantly reduced the sensory acceptability of products. Extrusion cooking of legume would allow reduction of antinutritional factor levels and improvement of nutritional quality at a cost lower than other heating systems (such as baking, autoclaving, etc.) due to a more efficient use of energy and better process control (Reimerdes, 1990). The effect of high temperature short time (HTST) treatment compared with other conventional processes on protein, phytic acid, condensed tannins, polyphenols, trypsin, chymotrypsin and α-amylase inhibitor activities and haemagglutinating activities in pea seed were investigated (Alonso *et al.*, 1998). Trypsin and chymotrypsin inhibitors and haemagglutinating activities in peas were more readily inhibited by extrusion treatment.

Extrusion cooking increases the availability of proteins or nutrients in the amaranth grain, and the available lysine remains the same as the raw material (Mendoza and Bresani, 1987). To increase protein content and nutritive value, various protein sources such as fish or peanut flour can be included in snack formulations prior to extrusion. Fish protein has an excellent nutritive value because it contains essential amino acids and is highly digestible (Haard, 1995). Moreover, fish is a good source of fat-soluble vitamins such as vitamin E, found in flesh, and vitamin A and D, found in liver (Exler and Weihrauch, 1976). Fish also contains several polyunsaturated fatty acids, which have desirable health benefits. In addition, the fish flavor is desirable in snacks produced for the international market.

Suknark *et al*. (2001) assessed the stability of tocopherols and retinyl palmitate in extruded snack products. They found that extrusion significantly reduced the content of tocopherols and retinyl palmitate in the final product. Effects of extrusion cooking on nutritional quality are ambiguous. Killeit (1994) carried out study on vitamin stability in extrusion cooking and fortification of extruded products with vitamins. As far as vitamins are concerned, depending on production parameters, mainly destructive effects are reported. Depending on the particular vitamin, considerable degradation can occur, especially in products with high sensory appeal. The nutritional quality of two blends of rice grits, one with soy grits and one with lupin flour, processed by extrusion, was evaluated on the basis of proximate analysis of fat, protein, carbohydrates and certain minerals (Ruales *et al*., 1988). The protein content was 12.6% for rice-soy and 15.3% for rice-lupin. The amino acid composition showed that the limiting amino acid in both blends was tryptophan.

2.3.14. Colors, flavors and additives

The color of a fabricated food has to be within an envelope of acceptance. In 1976, Commission Internationale de 1’ Eclairage (referred as CIE) addressed this issue by introducing L*a*b* color space (Lauro, 2000). In this space, L* indicates lightness, whereas the a* and b* are the chromaticity coordinates; a* is the red/green axis (“+” being toward the red and “-” being toward the green). Similarly b* is the yellow/blue axis (“+” being toward the yellow and “-” being toward the blue). This method of defining color exactly pinpoints the color in a three-dimensional color space. The L* value from 0-100 establishes the points white-to-black content. Consequently the L*, a*, b* color space, where the intersection of the three values of the perceived color and is most commonly used parameter to specify the color of the product.

Apruzzese *et al*. (2000) demonstrated the feasibility of using a fiber-optic equipped visible-near-infra-red (VIS-NIR) spectrometer to monitor both colour and composition in an extruder during the extrusion of yellow corn flour. In a statistically designed set of experiments with no added colourant, reflectance spectra were acquired and converted into CIE L a b colour coordinates. These values were shown to respond to changes in extruder screw speed, temperature and the interaction of temperature with screw speed. Screw speed had a direct effect on the colour of the extruded samples, because of the differences in shear stress and residence time. The effect of temperature is not simple because temperature affected both the physical properties of the polymer melt and the chemical reaction kinetics (Davidson, 1984). The viscosity of the melt increases exponentially with decreasing temperature (Harper *et al.*, 1971) resulting in higher shear stresses at lower temperature.

Colour measurements have been most precisely done using the Hunter Tri stimulus Color Difference Meter (Harper, 1981). Such meters allow color to be described in terms of L (total lightness), a* (+ = red, - = green), b* (+ = yellow, - = blue) and ΔE (total color difference). In using such meters, it is important that a standard color be specified and the meter set to that standard before each determination. Consumer acceptance and repeat purchase of extruded foods occurs because of their convenience, value, attractive appearance and desirable flavor. To enhance the last two attributes, some food coloring and flavoring materials are added as part of the ingredient mixture fed directly to the extruder, but most flavor addition occur after the product has been extruded.

The use of color in extruded foods can increase their appeal and consumer acceptance. A variety of food coloring agents are available to improve the appearance. There are two categories of food color additives, which are recognized as acceptable. Dyes which are soluble in water are also termed lakes. Unlike flavoring materials, the best place to add color to extruded foods is to the ingredients before extrusion (Kinnison, 1971, 1974). Although there are no limitations on the use of FD&C colorants in foods, good manufacturing practice limits their concentration to 300 ppm. Kinnison and Chapman (1972) performed a series of experiments with food colors added before the extrusion of corn grits in collet extruders. At 200^{o}C, FD&C Red No.2 and FD&C Blue No.2 faded, while other dyes were stable. Fading of color components is a common occurrence in extruded foods. Kinnison (1974) suggested that high temperatures over extended periods of time could be responsible for fading with certain colors. Proteins also react with food colors to cause fading. Metal ions and reducing sugars can react with dyes to cause fading. Expansion or puffing of the extruded product dilutes or fades the color but can be overcome by adding more color.

Although the Maillard reaction is primarily responsible for much of the color that develops when most foods are heated, the majority of the developed colored compounds remain uncharacterized (Ames, 1992). Ames *et al.* (1997) reported the extraction of some colored material from an extrusion cooked starch-glucose-lysine system-using methanol.

The starch may chemically or physically bind the colored material produced from glucose-lysine interactions and it may also react directly with lysine to give coloured compounds. Ames *et al.* (1998) analyzed the non-volatile Maillard reaction products formed in an extrusion-cooked model food system.

Sgaramella and Ames (1993) studied the colour development and control in extrusion-cooked foods. Mixtures of wheat starch, glucose and lysine, 96:3:1 and 92:6:2 (m:m:m) of three moisture contents (13, 15 and 18%) were extrusion cooked using two target die temperatures (125^o and 135°C). They observed that with an increase in die temperature or the amounts of glucose and lysine, or decreasing the moisture content, there was an increase in the colour intensity of the extrudates which resulted in decreased L* and increased a* and b* values. They also observed that expansion was much poorer at 18% moisture content when compared to 13 and 15%.

Bredie *et al.* (2002) studied the effect of temperature and pH on the generation of flavour volatiles in extrusion cooking of wheat flour. They observed an increase in levels of pyrroles, thiophenes, thiophenones, thiapyrans and thiazolines with increase in extrusion temperature. With increase in pH also most of the flavour volatiles tended to increase. Meat flavors have become increasingly important with the development of textured plant proteins used as meat analogs and extenders. Kinsella (1978) and Ritter (1978) have discussed the nature of meat flavors and their developments in the field. Ritter (1978) lists the major volatile components of meat flavor as a variety of alcohols, amines, aldehydes, ketones and phenolic acids. The production of artificial meat like flavouring agents has been enhanced by the use of hydrolyzed vegetable proteins and autolyzed yeast extracts which are mixtures of amino acids and peptides. Synthetic flavors also contain spices, salt, MSG, inosine-5'-nucleotide (IMP) and guanosine 5'-nucleotide (GMP) (Kinsella, 1978).

Under high temperature and pressure the flavor components can react with the food mixture changing their character and flavor. Krukar (1971) indicated that the rapid expansion leads to loss of flavour components dramatically, reducing the intensity in the finished extruded products. Blanchfield and Ovenden (1974) report that some progress has been made in using encapsulated flavors to protect them during extrusion but the numbers of flavorings available are limited. Kinnison and Chapman (1972) concluded that internal flavoring was not the answer to flavoring extruded foods but it could serve to enhance the flavor that was provided by surface coatings on extruded products. Surface applied flavours give an immediate taste sensation to the consumer and produce the greatest flavor response with the minimum of flavoring material.

Enrobing or surface coating is the most common method for the addition of flavor, color, and additives to extruded products. The common enrober is a horizontally mounted cylindrical drum, which rotates about its central axis. The drum is mounted on a slight incline so that the product advanced down the length of the enrober, as it is tumbled by the rotation of the drum (Harper, 1981).

2.4. Protein-polysaccharide interactions

Understanding the mechanism of protein- polysaccharide interaction will help in modification of extrusion process and properties of extrudate. Protein-polysaccharide interactions are not specific classes of interactions but are typical of the types of interactions that may occur between different types of polymer in solution (Ledward, 1994). The specific interaction between a protein and a polysaccharide during extrusion has been studied with alginate and soy model system. The addition of the alginate markedly reduces the extruder torque and product temperature in both single and twin-screw extrusion (Smith *et al.*, 1982; Berrington *et al.*, 1984; Imeson *et al.*, 1985). This effect was shown to be due to a significant reduction in viscosity (Berrington *et al.*, 1984). Among different polysaccharides (including guar gum, locust-beam gum, CMC, pectin and carrageenan) studied to elucidate the mechanism of protein-polysaccharide interaction, only alginate had a measurable impact on extrusion behavior at the 1% level. Oates *et al.* (1987 a, 1987 b) studied the phenomenon in more detail and convincingly demonstrated that heating soy in the presence of alginate, at temperatures similar to those experienced in extrusion processing, led to the formation of water, which would account for the decrease in viscosity observed during extrusion processing in the presence of this polysaccharide.

The degraded polysaccharides, unlike the native materials are able to undergo browning reactions with both glycine and lysine and thus it is possible that the reactant group is an anhydro end group formed by cleavage of the glycosidic bond. Further such groups are known to be highly reactive and able to undergo browning reactions with amino groups. Then the likely reactants on the protein would be the amino groups. Lysine is an obvious candidate but since this grouping is known to partake in such reactions irrespective of the presence of alginate, other groups are also presumably get involved. Oates *et al.* (1987 b) have presented convincing evidence that glutamic acid (or amide) groups are involved, since of a whole range of proteins tested, only soy and gluten showed significant formation of additional water in the presence of high M alginate. In soy and gluten most of the glutamic acid residues exist in nature in the amide form, and it is tempting to suggest that these are the reactive groups. This contention about the potential role of glutamate residues in reacting with accessible carboxy groups on the polysaccharide may be of more than academic interest, since it is well established that the glutamate-rich proteins (soy and gluten) are more amenable to texturization by extrusion than most other proteins (Ledward and Mitchell, 1988). The only other amino acid residue to show a significant decrease on heating was lysine; the decrease in this case was independent of the presence of alginate. Though the detailed chemistry of these reactions has yet to be elucidated, the covalent linkages established during the reactions, which produce water as a by-product, may be of key importance indicating the texture of extruded proteins (Ledward and Mitchell, 1988).

Since 1990 several workers (Kobayashi *et al*., 1990; Kato *et al*., 1990; Kato and Kobayashi, 1991; Dickinson and Galazka, 1991) have described the preparation of covalent protein-polysaccharide complexes prepared by controlled ‘dry’ heating of certain proteins, β-lactalbumin (Dickinson and Galaska, 1991), soy (Kobayashi *et al*., 1990), and ovalbumin (Kato *et al*., 1990) and a polysaccharide (dextran and PGA). These soluble complexes are formed by a Maillard reaction between the two biopolymers and possess excellent functional, especially emulsifying properties. The actual nature of the covalent linkages(s) has not yet been determined, but since the molecular weight of the ovalbumin-dextran complexes are in the range of 130,000-230,000. Kato and Kobayashi (1991) suggest that the bond forms between the reducing end group of the dextran molecule and the ε-amino group of lysine so that only one or two moles of dextran can bind per mole of protein. The low water content of the system presumably ensures that the amino group is in the reactive undissociated form. There are few studies on interaction of corn starch (Shim and Mulvaney, 2001) and cassava starch (Aguilera and Rojas, 1996, 1997; Aguilera and Baffico, 1997) with whey protein.

There is a further need to study protein-polysaccharide interaction with different systems, especially from fish so as to evolve appropriate unit operations for enhancing the extruded product quality.

2.5. Gelatin

Gelatin is a protein derived by hydrolytic degradation of collagen, the principle component of white fibrous connective tissue (Gilsenam and Murphy, 1999). The source and type of collagen will influence the properties of the resulting gelatins. The basic molecular unit of collagen is a triple helical rod. This consists of three alpha chains arranged in a left-handed axis, with the whole structure wound into a right-handed super helix.

Gelatin is a protein compound which in itself may be considered a highly – digestible dietary food ideal as a complement in certain types of diet (Johnston-Banks, 1990). Gelatin can be used as an ingredient to enhance the elasticity, consistency and stability of food products. Collagen can be converted into a soluble gel forming material, for use either as food grade gelatin or as glue. Gelatin has played a major role in the development of colloid chemistry and the gel formation; film formation and surface properties of gelatin have been studied extensively.

The largest use of gelatin is in gel desserts. The estimated world usage of gelatin is 200, 000 metric tons/year (Choi and Regenstein, 2000). Gelatin is well known in everyday life for its ability to form soft gels at room temperature, which have been successfully used in food, pharmaceutical and photographic applications.

2.5.1. Raw material for gelatin production

The main raw material for gelatin production is skin and bones from bovine and porcine source. The raw material, which is rich in collagen, is made use of in gelatin preparation. Recently, problem appeared on using the collagen from beef with the outbreak of disease and epizooties of the cattle in Europe. This has strongly decreased the availability of raw material as the suspicion thrown on the safety condition in extracting gelatin (Djabourov *et al*., 1993). Hence, there has been interest in producing non-beef and non-pork gelatin due to Bovine Spongiform Encephalopathy (BSE) crisis as well as religious and social reasons. Production of gelatin from pig's skin is not acceptable for religious reasons (Judaism and Islam) and gelatin from beef is acceptable only if it has been prepared according to the religion requirement. On the other hand use of skin and bones of fish for gelatin production will be acceptable to all religious sects and hence can form an ideal raw material (Choi and Regenstein, 2000).

New sources of raw material for gelatin production are being explored, among which gelatins extracted from skin of fish and underutilized fish is gaining importance (Nagai and Suzuki, 1999). As the availability of fish processing waste is huge, which can be made use for gelatin preparation.

2.5.2. Gelatin from fish processing waste

The waste from fish processing after filleting can account for as much as 75% of the material used (Shahidi, 1994). This waste is an excellent raw material for the preparation of high-protein foods, besides helping to eliminate harmful environmental aspects and improve quality in fish processing (Gomez Guillen *et al*., 2002). About 30% of such waste consists of skin and bone with high collagen content. Heat denaturation of collagen produces gelatin. Fish skin is potentially a valuable source for gelatin, despite the problems associated with eliminating fish odor from products.

Even though several groups have studied the physico-chemical properties of fish gelatins (Norland, 1990; Leuenberger, 1991; Choi and Regenstein, 2000; Gilsenan and Ross-Murphy, 2000), scanty information is available on the gelatin extraction procedures from skins of different marine species. The data available are from studies on tilapia, conger eel, arrow squid, cod and megrim (Grossman and Bergman, 1992; Kim and Cho, 1996; Gudmundsson and Hafsteinsson, 1997; Montero and Gomez-Guillen, 2000).

Skins from tropical fish species such as tilapia, have been described as an optimal raw material for gelatin production (Grossman and Bergman, 1992; Holzer, 1996). Fish gelatin is seldom used and not mass-produced due to the dark color and fishy odor. The processing and functional properties of fish gelatin have been studied from tilapia (Choi and Regenstein, 2000). The properties of fish gelatin derived from lumpfish, tilapia, conger, squid, megrim and cod have been evaluated (Osborne *et al*., 1990; Grossman and Bergman, 1992; Jamilah and Harvinder, 2002; Kim and Cho, 1996, Gudmundsson and Hafsteinsson, 1997; Montero and Gomez-Guillen, 2000).

The main drawback of gelatin from fish is that they are less stable and lack of desirable rheological properties than gelatins from land mammals, which seriously limits their field of application (Leuenberger, 1991). This has been largely related to the considerably lower number of proline rich regions of the collagen or gelatin molecule in cold-water fish, than in warm-blooded animals (Ledward, 1986; Norland, 1990).

Moreover, it has also been described that the total Gly-Pro-Hyp sequence content is one of the main factors affecting collagen thermo stability (Burjandze, 2000).

2.5.3. Extraction methods

Extraction and characterization of gelatin from fish skin have been studied (Gudmundsson and Hafsteinsson, 1997; Choi and Regenstein, 2000; Montero and Gomez-Guillen, 2000; Gomez-Guillen and Montero, 2001; Gomez-Guillen *et al*., 2002; Gudmundsson, 2002; Cho *et al*., 2005). Different methods for manufacturing gelatin provide distinct differences in the properties of gelatin. One method involves extraction of gelatin at a neutral or slightly acid pH, after pre-treatment of raw materials with cold alkaline solution. Collagen, when subjected to the acid or alkaline hydrolysis is broken down irreversibly and forms a viscous solution in water, which forms a gel on cooling. The chemical composition of this gelatin is in many respects closely similar to that of the parent collagen (Eastoe and Leach, 1977). The first step before extracting gelatin from the raw material is the hydrolytic breakdown of covalent bonds and conversion of regular structure of collagen to that of gelatin and their release of gelatin fragments from the fibrillar structure. The second step involves thermal denaturation, which breaks down collagen irreversibly. Denaturation of soluble collagen at 40^{o}C takes place by destroying the triple helical structure of collagen to produce one, two or three random chain gelatin molecules (Flory and Waver, 1960). In this process, the hydrogen and probably electrostatic bonds and triple helix of collagen structure become free from each other and pass into solution as random coils. The properties of gelatin, molecular weight, number of each kind of amino acid residues and number of polypeptide chains are highly dependent on the position of the breaks.

2.5.4. Properties of gelatin

The quality and properties of the gelatin depends on the composition of the raw materials and the factors including the species, breed, age, feeding habit of the animal and storage condition of raw material (Hinterwaldner, 1977). One of the most important factors in the quality of fish gelatin is the environmental condition of the fish species. In general, collagen and gelatin, prepared from low temperature fish species contain a lower amount of proline and hydroxy proline and have a lower number of hydrogen bonds and lower melting point than species from warm water temperature environment (Arnesen and

Gildberg, 2002). Gelatin contains nearly 30% glycine, which is the most dominant amino acid (Rother, 1995; Sarabia *et al*., 2000; Gomez-Guillen *et al*., 2002; Arnesen and Gildberg, 2002).

As reviewed by Stainsby (1987) and Johnston-Banks (1990) the physical properties of gelatin depends on the amino acid composition, but also on the relative content of α-chains, β- or γ- components and higher molecular weight aggregates, as well as on the presence of lower molecular weight protein fragments.

Although the commercial value of gelatin is principally based on gel strength, a wide range of physical and chemical tests may be carried out on gelatin and gelatin products. The most important physical properties of gelatin are gel strength and viscosity (Wainwright, 1977). Gelatin with low viscosity forms a short and brittle gel whilst gelatin with high viscosity gives a tougher and stronger gel; commercially, high viscosity gelatin is preferred and has a higher price. Gelatin is classified into 3 kinds, low bloom (150 and below), medium (150 – 220) and high bloom (over 220 upto about 300) (Johnston-Bank, 1983).

The functional property of gelatin is related to their chemical characteristics. The gel strength, viscosity, setting behavior and melting point of gelatin depend on their molecular weight distribution and the amino acid composition (Johnston-Banks, 1990). Studies conducted on warm water fish gelatin showed that they have better functional properties than gelatin from cold-water fish species (Gilsenan and Ross-Murphy, 2000; Grossman and Bergman, 1992). This has been attributed to their higher content of imino acids in gelatin from warm water fish. The quality of gelatin depends on its physicochemical properties, which are greatly influenced not only by the species or tissue from which it is extracted, but also by the severity of the processing method (Johnston-Banks, 1990). The quality of a gelatin for a particular application depends largely on its rheological properties (Stainsby, 1987).

The quality of a food grade gelatin depends largely on its thermal and rheological properties. Good rheological properties are required for many applications and could be attained by using gelatin-modifying material (Sarabia *et al*., 2000). One possible means of manipulating the characteristics of a given gelatin is to trigger interactions by the addition of solutes, like salts (Elysee-Collen and Lencki, 1996). It has been stated that the effect of salt concentration on protein stability is very ion specific, with stabilizing effects typically following the Hofmister series (Von Hippel and Wong, 1962). The effect of different salts on the rigidity or melting temperature of animal gelatin has been understood (Harrington and Von Hippel, 1961).

III. MATERIALS AND METHODS

3.1. Materials

Fresh ribbonfish (*Trichiurus spp.*) and bull's eye fish (*Priacanthus spp.*) caught by trawl net along the coast off Mangalore, India, were used for the study. Immediately after harvest, fish were washed in fresh water and iced in the ratio of 1:1 (fish: ice) and transported to the laboratory. The whole fish was washed in chilled water prior to dressing. Head and entrails were removed manually and washed with chilled water. Separated meat from these fishes was used for the preparation of raw material for extruded products.

Rice flour, wheat semolina and ragi flour were procured from the local market for extrusion process.

3.1.1. Chemicals

All the chemicals and reagents used in the present study were either AR or GR grade. Sulfuric acid, hydrochloric acid, citric acid, acetic acid (glacial), acetone, petroleum ether, boric acid were procured from Qualigens fine chemicals, India. Sodium acetate trihydrate, (Mallinckrodt), Triethylamine (TEA – Waters Part Number 88121 or equivalent), Glacial acetic acid (J.T. Baker), Acetonitrile (Burdick and Jackson), Water (Milli-Q quality or equivalent), Disodium hydrogen phosphate (Na_2HPO_4, Mallinckrodt), Phosphoric acid (J.T. Baker), Methanol, Sodium Acetate (IM), derivatising agent (PITC), Amberlite mixed resin were obtained from Fischer chemicals UK. Disodium hydrogen phosphate, sodium dihydrogen phosphate, copper sulphate, potassium sulphate, sodium chloride, ammonium per sulphate, ammonium sulphate, sodium hydroxide and selenium dioxide were procured from E.Merck (India) Limited, India. Bromophenol blue, methyl red, methylene blue, and glycerol were procured from Ranbaxy laboratories Limited, India. Sodium Dodecyl sulphate, TEMED, acrylamide, bis-acrylamide (N, N'-methylene-bis-acrylamide), 2-mercapto ethanol, Trizma base (tris [hydroxyl methyl] amino methane), Coomassie brilliant blue and standard markers were obtained from Sigma chemical Co., USA.

3.1.2. Equipments

The equipments used in the present investigation include a twin-screw extruder, Differential scanning calorimeter, Raman spectrometer, HPLC amino acid analyzer, Varian gas chromatograph, Texture analyzer, Colorimeter, Controlled Stress Rheometer, Phase contrast microscope, Vacuum flash evaporator, Hot air oven, Soxhlet apparatus and other analytical equipments.

3.1.2.1. Extruder

The extruder used for the present study is a twin-screw extruder (Basic Technologies, Pvt., Ltd., Calcutta, India) (Fig. 2) with co-rotating three start screws (Fig. 3). The three-start screw of the extruder was 29.7 mm in diameter and 350 mm long. The pitch of the screw was 75 mm with a flight depth of 3.5 mm

The drive system of the extruder is provided with ABB Frequency Converter to control the RPM precisely according to the need of process. Since this type of drive generates torque gradually, a bypass switch (installed at the side of control desk) is provided to directly apply the drive from motor, which gives the sudden application of torque necessary to clean the barrel from burnt-out products, by the “inching device”. The main drive is provided with a 5 HP Motor (400 V 3 Ph. 50 Hz). The out-put shaft of worm reduction gear is provided with a Torque limiter coupling. This device consists of a Torque limiter and a roller chain type coupling. The torque limiter is a protective device that limits torque transmitted by the out-put shaft of worm reduction gear. The torque limiter utilizes ‘spring loaded friction surface’, when there is overload, the friction surface slips and some smoke may come out if there is any oil contamination.

The out-put of the coupling rotates the input shaft of Duplex gear which has two out-put shafts to rotate two screws inside the barrel in co-rotating fashion. The barrel of the extruder receives the feed from a co-rotating feeder (variable speed). The knob of the controller controls the rated capacity of the feeder. The barrel is provided with two electric and band-heaters and two water cooling jackets. A temperature sensor is fitted on the front die plate, which is connected to temperature controller placed on the panel board. The second band heater is controlled by temperature controller (SUNVIC, UK).

The die is fixed by a screwed nut tightened by a special wrench provided. The automatic cutting knife is fixed on a rotating shaft of knife drive assembly. The cutter is driven by a variable speed DC motor, which is controlled by a knob placed on the panel board. The Automatic cutter assembly is covered by a removable hinged guard.

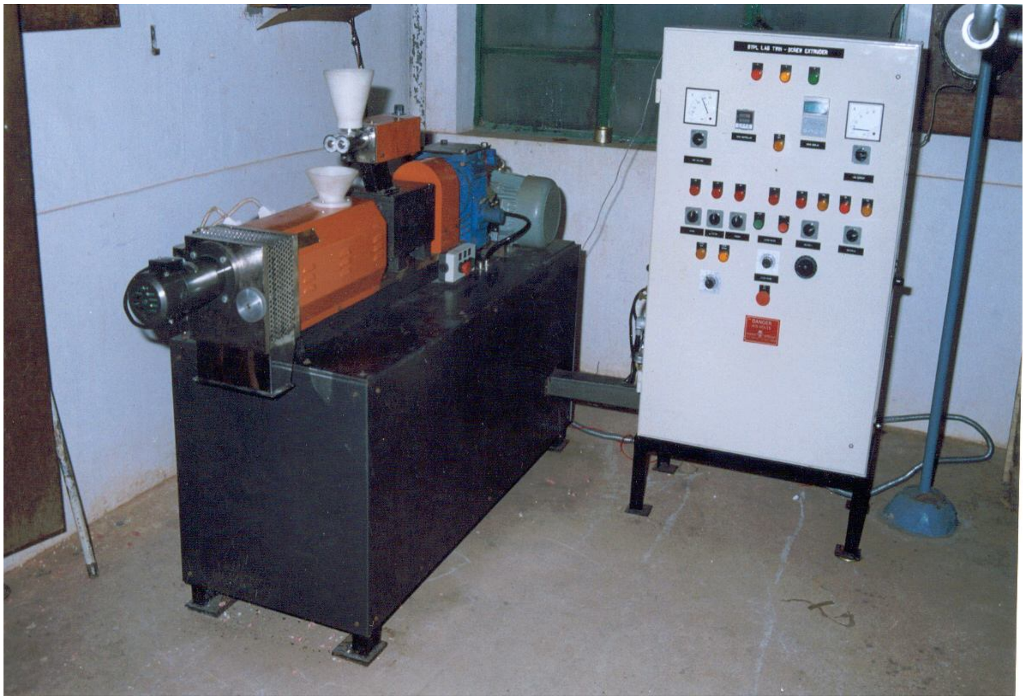

Fig. 2: The full assembly of twin-screw extruder used for the study

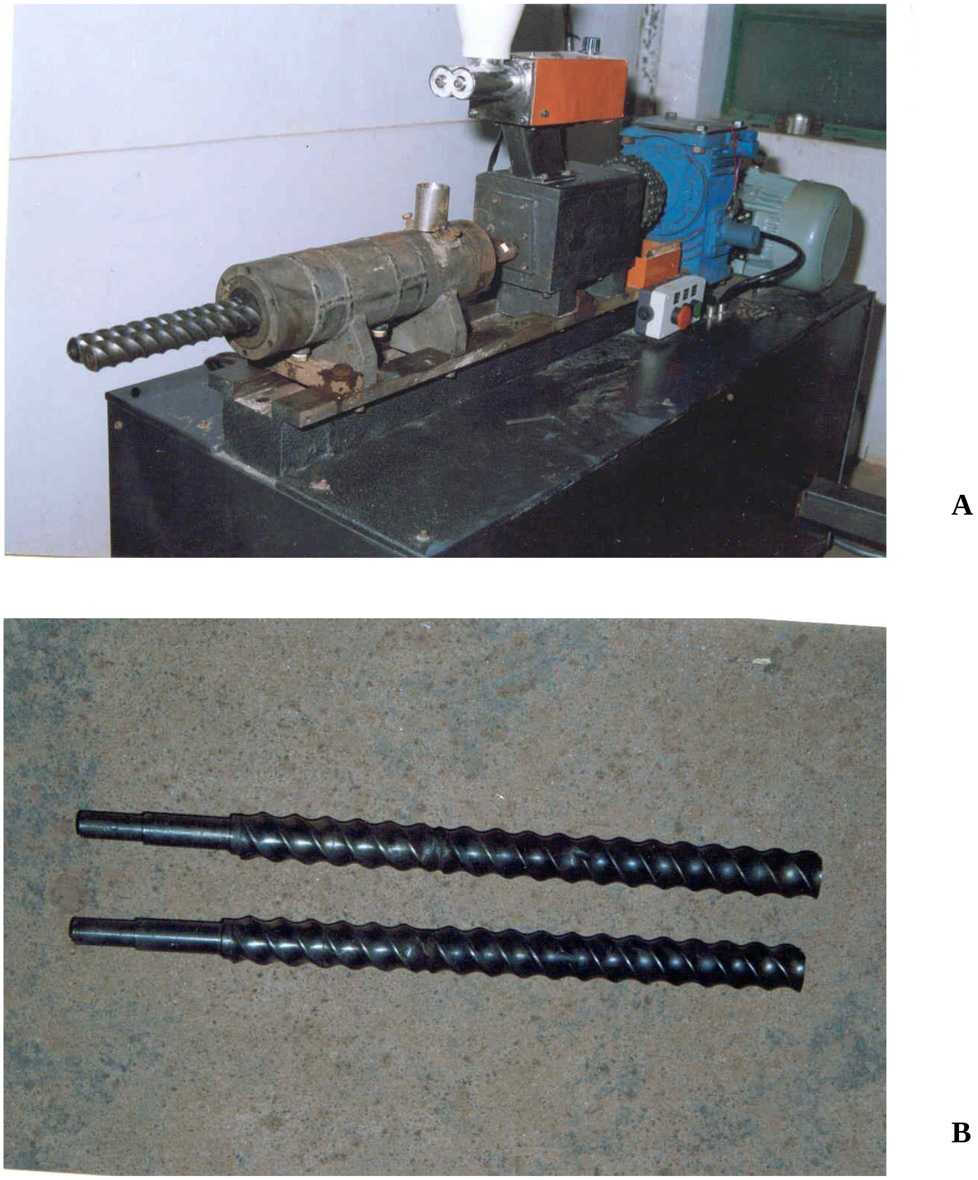

Fig. 3 A: Barrel of the extruder with screw exposed

B: Dismantled screw of the extruder

3.2. Methods

3.2.1. Preparation of extruded products

3.2.1.1. Raw material preparation

Extruded products were prepared using a blend of rice flour/wheat semolina/ragi flour and fish mince (Ribbonfish/Bull's eye fish). The initial moisture content of rice flour, wheat semolina and ragi flour was 5.5%, 7.5% and 11.37% respectively. The flours were mixed with fish meat at 10% and 20% (w/w) level. The final moisture content of the mixture with 10% (w/w) of fish mince was adjusted to 15% by addition of water. With the addition of 20% fish mince moisture will be slightly above 15% (16.5%). Appropriate controls were prepared wherein, no fish mince was added and the moisture content was kept at 15% by addition of water.

Ribbonfish and bull's eye fish mince with moisture content of about 78.0% and 78.8% respectively were mixed with rice flour, wheat semolina and ragi flour separately and the final moisture content was adjusted to 15%. The blend was then mixed with sodium chloride at 2.5% (w/w) level. The mixture was thoroughly mixed and then sieved through a specific mesh to get uniform particle size. The sieved mixture was then kept for equilibration time of 30 minutes. After equilibration, once again the sample was sieved and kept ready for extrusion. The fish mince-flour mixture was used to prepare extruded products as a function of barrel temperature, screw speed and die diameter. Feeding rate of flour - fish mince mixture was adjusted to 120 g/minute (7.2 Kg/hr).

3.2.1.2. Extrusion process

The scheme used for the production of extruded is given in Fig. 4.

Extruded products as a function of barrel temperature

Extruded products from flour (rice flour, wheat semolina and ragi flour) with added fish (ribbonfish/bull's eye fish) at various levels (10% and 20%) were prepared as a function of barrel temperature. Barrel temperature at the expansion section was maintained at 70^{o}, 90^{o} and 120^{o}C respectively. Screw speed was maintained at a speed of 350 RPM

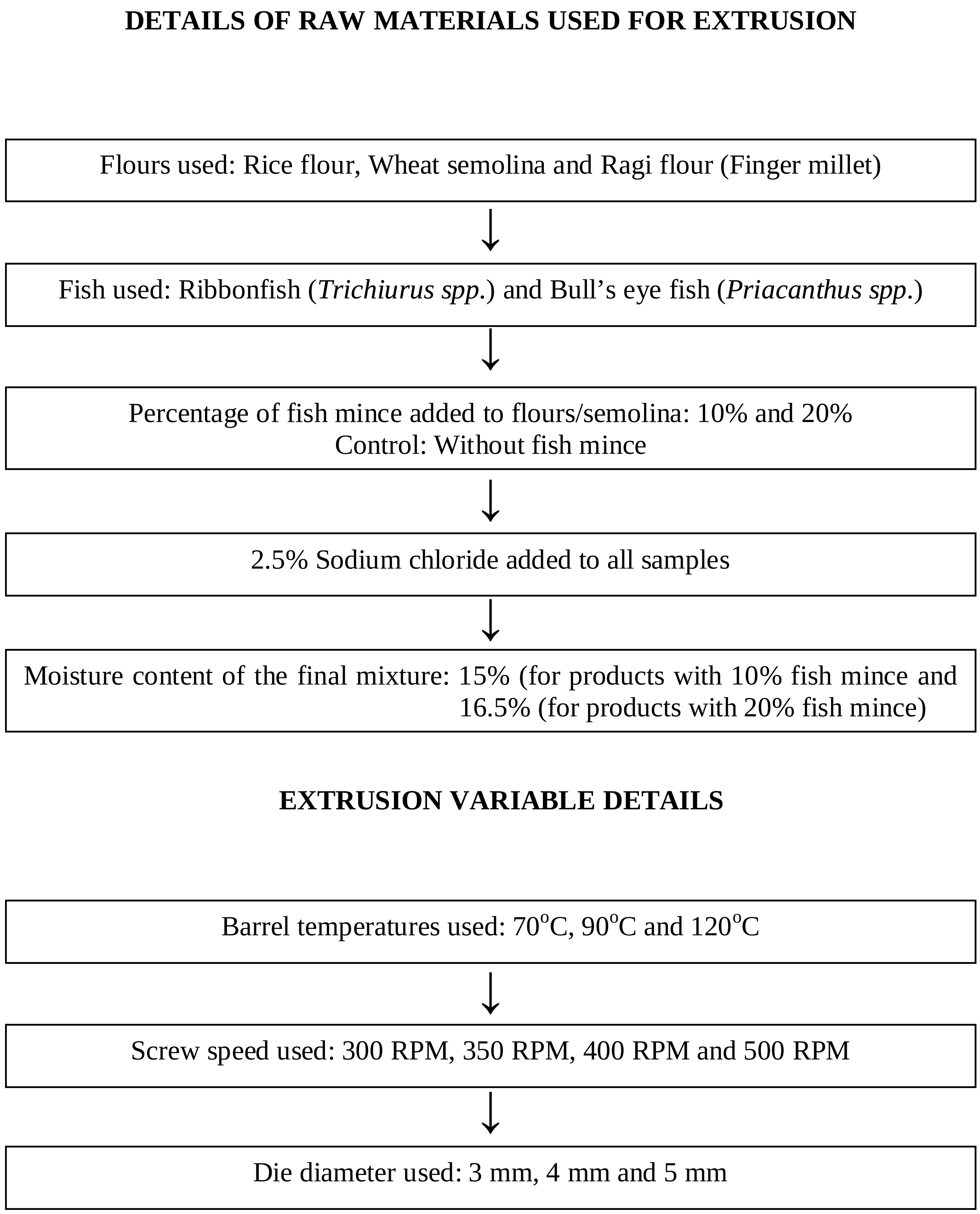

Fig. 4: Schematic representation of extrusion variables and raw materials used for extrusion using twin-screw extruder

and the die diameter used was 4 mm. At 120°C, only samples with 10% level fish mince has been carried out.

Extruded products as a function of screw speed

In order to find the effect of screw speed on the quality of extruded products, products were prepared from the three flour materials with 10% ribbonfish mince. Extrusion was carried out at 300, 400 and 500 RPM at a constant barrel temperature of 90°C and die diameter of 4 mm.

Extruded products as a function of die diameter

Extruded products were prepared from a mixture of flour (rice flour, wheat semolina and ragi flour) and ribbonfish mince (10% w/w) using different die diameters. The die diameters used were 3 mm, 4 mm and 5 mm. Extrusion was carried out at a constant barrel temperature of 90°C and screw speed was maintained at 350 RPM.

3.2.2. Gelatin preparation

Fresh skins of bull's eye fish were used for gelatin preparation following the method of Gudmundsson and Hafsteinsson (1997). Skin was separated from fish flesh and cleaned, washed several times with tap water and was dried in between two pieces of Whatman filter paper. Dried clean skins were cut into 2 x 2 cm pieces. Sodium hydroxide solution 1000 ml (1.5 g NaOH in 1000 ml distilled water) was added to 140 g of skins, shaken well and slowly stirred at 20°C for 40 minutes. The skin pieces were separated and rinsed with water. This procedure was repeated three times. Next the skins were treated, in the same way, but with 1000 ml sulphuric acid solution (1.5 ml H_2SO_4 in 1000 ml distilled water) and rinsed with water. This procedure was also repeated three times. Finally the skins were treated with 1000 ml citric acid solution (7 g citric acid in 1000 ml distilled water) with subsequent rinsing; this was also repeated three times. Fish skin was then extracted with distilled water in a 45°C water bath overnight (17 hrs) without stirring. The mixture was filtered using Buchner funnel with Whatman paper No. 4. The resultant filtrate was evaporated in a rotary evaporator at 45°C to reduce the volume to 1/10th the original volume.

To 25ml of # 5% gelatin solution 1g of amberlite mixed resin was added and stirred well. The mixture was heated at 65°C, for 30 minutes with constant stirring. The conductivity of the solution was measured using a conductivity meter (Suntex Conductivity Meter SC-170, Taiwan). If the measured conductivity was found to be above 50µS/cm, further 1 g of resin was added and heated at 65^{o}C for another 30 minutes, with constant stirring. The process was repeated till the measured conductivity value was below 50µS/cm. The solution was decanted and filtered through Whatman paper No. 4. The pH of the filtrate was measured at 30^{o}C and adjusted to pH to 6.0 by using 0.1M sulphuric acid.

3.2.3. Proximate composition

Proximate composition of fresh fish (ribbonfish/Bull's eye) was determined. Meat was separated and macerated well prior to proximate analysis. Proximate composition of the rice flour, wheat semolina and ragi flour were also determined (AOAC, 1995).

Moisture, protein, fat and ash content of extruded products and bull's eye fish's skin were estimated by the method as described in AOAC (1995). Moisture and protein content of gelatin was also estimated.

Moisture

Moisture content of the samples was determined according to the method of AOAC (1995). A known quantity (about 5g) of the sample was taken in moisture bottle, dried in hot air oven at 105^{o}C for 10-12 hours and cooled in desiccator to a constant weight. The weight loss during this drying process is expressed as the moisture per 100g of sample.

Total Nitrogen

Total nitrogen was estimated by Kjeldahl method according to the method of AOAC (1995). About 1 g of sample was digested with 10ml concentrated sulphuric acid and a pinch of digestion mixture (Potassium sulphate: copper sulphate: selenium dioxide in the ratio of 100:10:2.5) until the digest becomes clear or colorless. After cooling the volume was made up to 100ml with distilled water. From this solution, 2 ml was taken for distillation along with 15ml of 40% sodium hydroxide in Kjeldahl distillation apparatus. The ammonia liberated during distillation process was absorbed in 2% boric acid containing mixed indicator [methyl red (0.6%): methylene blue (0.6%):: 2:1] and titrated against standard N/140 hydrochloric acid.

The nitrogen equivalence of standard hydrochloric acid was determined using ammonium sulphate solution containing 1 mg nitrogen/ml. The standard ammonium sulphate solution was distilled using Kjeldahl apparatus. The distillation was carried out and the amount of hydrochloric acid consumed to titrate was noted. From this, equivalence of hydrochloric acid for 1 mg nitrogen was calculated. One ml of N/140 HCl was equivalent to 0.1169 mg nitrogen. Crude protein content was determined by multiplying total nitrogen by a factor of 6.25. In case of crude protein content of gelatin the multiplying factor was 5.5.

Crude fat

Crude fat was determined using Soxhlet apparatus according to the procedure of AOAC (1995). About 1 g of sample was taken in a thimble and placed in a Soxhlet extraction unit using petroleum ether (60-80^{o}C) as solvent. The extraction was carried out for 16 hours. The solvent with dissolved fat in the receiver was evaporated and the residual solvent was removed by keeping in hot air oven at 60^{o}C and cooled to a constant weight. The crude fat content was expressed as percentage.

Ash

Ash content of the sample was determined by method as described in AOAC (1995). About 1 g of moisture free sample was taken in a pre-weighed silica crucible. Preliminary ashing was done by slow heating on flame to allow smoking off fat without burning. Once the smoke stopped evolving from the sample, it was incinerated in a muffle furnace (SUNVIC, UK) at 500^{o}±10^{o}C for 5 hours. The crucibles were removed and cooled in desiccator and weighed. Ash content was calculated from the weight difference of crucible and expressed as percentage on wet weight basis.

3.2.4. Aminoacid composition

Aminoacid composition of two selected fish species (0.2 g), and the gelatin extracted from bull’s eye fish skin was determined after derivatization with phenylisothiocyanate (PITC) according to the Waters Pico-Tag method as described by Bidlingmeyer *et al*. (1984) using the Waters Pico-Tag HPLC amino acid analyzer (Water Model 712 WISP, Waters, Watford, Herts., UK). Aminoacid composition of the extruded rice flour - ribbonfish / bull’s eye fish mince (added at a level of 10% and 20% w/w) mixture processed at a barrel temperature of 90^{o}C, was also determined. Weight of the sample taken for aminoacid analysis was 0.1 g of extruded product.

Sample (0.1/0.2 g) was weighed into screw cap pyrex vials, washed with 6N HCl, rinsed with deionised water and dried. 10 ml of 6 N HCl was added into the vials and flushed with nitrogen and quickly capped. Vials were then kept inside oven at 110^{o}C for 24 hours. To 20µl of the sample/ Standard, 10µl drying solution (200µl Methanol, 200µl Sodium Acetate (IM), 100µl Triethylamine) were added and vortexed and dried. Derivatisation was carried out by adding 20µl derivatising agent (20µl PITC, 140µl Methanol, 20µl Triethylamine, 20µl Water) and dried for 10 minutes at ambient temperature. Further drying was continued by adding 10µl of methanol and vortexing. Reconstitution of dried material was carried out in 100µl eluent A (Sodium Acetate Trihydrate; pH = 6.4 (940 ml) + 60 ml Acetonitrile) and injected to the column. Column used was Nova-Pak C-18, 3.9 x 150 mm column, Waters. Flow rate was maintained at 1-1.5 ml/min and concentrations of eluted fractions were monitored at a wavelength of 254 nm.

3.2.5. Fatty acid composition

Fatty acid composition of fat extracted from ribbonfish and bull's eye fish meat were determined. Transesterification was performed according to Schmarr *et al.* (1996). The prepared fatty acid methyl esters (1 µl) were then injected into a Varian gas chromatograph, Series 3600, with a hydrogen flame ionization detector using helium as a carrier gas. An initial temperature of 180^{o}C for 4 minutes was raised to 250^{o}C at a rate of 4^{o}C/minute. Standard reference fatty acids were also chromatographed to identify the individual fatty acids.

3.2.6. Differential Scanning Calorimetry

The thermodynamic parameters of the samples were examined using a DSC VII calorimeter (Setaram, Lyon, France). The calorimeter include a detector micro DSC VII (-10^{o} upto 120^{o}C), a microprocessor for calorimeter operation and control of the gas and vacuum circuit, a gas circuit to protect, a Pentium 75 (TM) microcomputer, a multitasking and multimodulus software under Windows (TM) and a printer. To avoid steam condensation in the calorimeter wall especially at low temperature, a constant sweeping of inert gas (nitrogen) is used. The water circulation ensures the evacuation or supply of heat to the two thermostatic walls. The calorimetric block of the Micro DSC VII is composed of metallic cylinder with a high thermal conductivity. Two blocks machined cavities take the measurement and reference experimental vessels. The vessels take 2 plates on the top as thermal buffers for a set of cylindrical covers. A peltier effect thermo element is placed between the block and the chamber to evacuate heat toward the intermediary chamber. The standard batch vessel is composed of a cylinder of 6.4 mm of internal diameter and useful height equivalent to 19.5 mm for the sample. The useful volume for the sample is equal to 1 cm^3.

The thermodynamic parameters of the fishes (Ribbonfish, Bull’s eye fish) and the flours (rice, wheat semolina, ragi) were examined using a DSC VII calorimeter (Setaram, Lyon, France). Flour materials were mixed with water to get a 10% (w/v) solution and this is loaded to the sample container. Water was kept as reference for all the samples. Heating rate was 0.5^oC/min from 10^oC to 90^oC. Heat absorbed or released by the sample results in either endothermic or exothermic peaks as function of temperature. For fish meat, the temperature reached when half of the sample is denatured is referred to as the transition temperature and was measured at the tip of the peak (T_m). The energy required to denature the sample, the enthalpy change (ΔH), was measured by integrating the area under the peak using the Setaram DSC software. The onset temperature (T_o) and peak gelatinization temperature (T_m) of the flour samples were measured from the endothermic curve in the thermogram. The energy required for gelatinization of flours was measured by integrating the peak area using the software.

3.2.7. FT-Raman spectroscopy

The Perkin Elmer Fourier-Transform (FT) near infrared (NIR) Raman spectrometer (System 2000) is a system that can collect both Raman and NIR spectra, using different sample chambers. The front-center chamber is used for the NIR path. The rear left chamber is attached to a continuous wave Nd.YAG (Neodymium depedyttrium aluminium garnet) laser operating at 1.064 µm (9398.5 cm^{-1}). Within the chamber, the laser beam emerges through an aperture and is steered by a mini prism attached to the collecting lens onto the sample. The beam comes to a gentle focus (c.a.100 µm) about 50 mm from the prism. The scattered light is collected (180^o back scattering) by the collecting lens and Raman components passes through set of low-pass filters into the spectrometer that produces an inter ferogram, detected on an InGaAs detector. The spectrometer is controlled by PC using a proprietary PE software package (Spectrum-V3).

Frozen fish (Ribbonfish and Bull's eye fish) samples were thawed and the meat was taken in 7 ml glass containers (FBG-Anchor, Cricklewood, London) on a Perkin-Elmer system 2000 FT-Raman spectrophotometer with excitation from a Nd:YAG laser at 1064nm. Starchy materials (rice, wheat and ragi) were also analysed both in native and gelatinized state using Raman spectrophotometer. Frequency calibration of the instrument was carried out using the sulfur line at 217 cm^{-1}. Triplicate analysis was carried out. The relative intensity has been calculated as the mean of the three trials. Laser power was set at 1500 mW for the fish sample and 800 mW for the starchy samples. The spectra were an average of 64 scans which was baseline corrected and smoothed. Gelatinization of the starchy materials has been carried out by heating the sample at 80^{o}C for 30 minutes. For gelatinized samples the spectra were an average of 128 scans. The spectra of fish were normalized to the intensity of the phenylalanine band at 1004 cm^{-1} (Howell and Li-Chan, 1996) and the spectra of the starchy materials were normalized to the intensity of the band at 480 cm^{-1} (Dupuy and Laureyns, 2002). The recorded spectra were analysed using Grams 32 (Galactic Industries Corp., Salem, NH) software. Assignments of the bands were done based on the literature (Schuster *et al.*, 2000; Howell and Li-Chan, 1996; Li-Chan *et al*., 1994; Careche *et al*., 1999) (Table 1).

3.2.8. Phase contrast microscopy

Changes in the physical structure of flour samples before and after gelatinization has been studied using Transmitted light microscope – Leitze (Leitz-laborlux D) with eyepiece power of 10x and objective (160/0.17, PLAN, 40/0.65, Phaco 2) power of 40x. Gelatinization of the starchy materials has been carried out by heating the sample at 80^{o}C for 30 minutes. The samples were placed on acetone cleaned microscope slides and covered with a cover slip to prevent dehydration. The slides were then viewed under the microscope. The microscope was attached to a Wild MPS 05 system comprising a camera and an exposure meter set.

Table 1. Peak assignment for starch samples

Wavenumbers cm^{-1}	Assignments of peaks	Ref.
426	Skeletal modes of carbohydrate rings (C-C-O)	Schuster *et al*., 2000
480	Skeletal modes	Schuster *et al*., 2000
Below 800	C-C and C-O deformations and skeletal breathing modes	Vandenabeele *et al*., 2000
947	α-1,4- glycosidic linkage	Schuster *et al*., 2000
950-800	C-O-C sugar ring vibration	Vandenabeele *et al*., 2000
1057	(C-C) + (C-O-H) modes	Schuster *et al*., 2000
1082	(C-O-H)modes	Schuster *et al*., 2000
1128	(C-O) and (C-O-H) modes	Schuster *et al*., 2000
1200-950	C-C and C-O symmetric stretching	Vandenabeele *et al*., 2000
1370-1380	(CH_2) scissoring	Schuster *et al*., 2000
1633	Water	Schuster *et al*., 2000
2908	(C-H)	Schuster *et al*., 2000
3213	(O-H)	Schuster *et al*., 2000

3.2.9. Rheological analysis

Small deformation test of flour/semolina solutions

Dynamic viscoelastic behaviour (DVB) of solutions (10% w/v) of rice flour, wheat semolina and ragi flour in the temperature range of 20^o-90^oC and cooling back to 20^oC was measured using a Rheometrics Constant Stress 200 rheometer under oscillatory mode with a 4 cm parallel plate geometry. 10% solutions of the flour samples were used for DVB measurement and the sample was surrounded by silicone oil to prevent evaporation of solvent. The gap between measuring geometry and Peltier plate was adjusted to 0.3 mm. The applied stresses for rice flour, wheat semolina and ragi flour were 0.13 Pa, 1.8 Pa and 0.15 Pa respectively. Applied stress was compared with the resultant strain. The results of such measurement were expressed as the storage modulus (G') and loss modulus (G'').

Protein-polysaccharide interaction

In order to study protein-polysaccharide interaction, Dynamic viscoelastic behaviour (DVB) of flour-ribbonfish mixture has been carried out as a function of time period at different temperatures (60^oC, 70^oC and 80^oC) using a Carri Med Controlled Stress Rheometer (CSR-500, Carri-Med, Surrey, UK.) under oscillatory mode. The proteins from fresh ribbonfish meat were extracted using extraction buffer (EB) and the concentration of protein used for the study was 10 mg/ml. Flour samples (rice flour, wheat semolina and ragi flour) were mixed with protein solution and the concentration of flour used was 20% (w/v). The measuring geometry used was 2 cm parallel plate. The gap between measuring geometry and peltier plate was adjusted to 200 µm. The gap was set manually at 70^oC using the micrometer provided with the system. Time sweep was carried out for 30 minutes at 60^oC, 70^oC and 80^oC. The applied stress of 20 Pa was within the viscoelastic region. The linear viscoelastic region was determined by a torque sweep with a frequency of 1 Hz. Measurements were made by applying a small amplitude oscillation (0.0005 Rad) with a frequency of 1 Hz. Applied stress was compared with the resultant strain. The results of such measurement were expressed as the storage modulus (G′) and loss modulus (G″). An average of three replicates was used for plotting the results.

Flow behaviour of gelatin from bull's eye skin (Shear Stress Sweep)

The flow properties of gelatin were measured as a function of temperature using Controlled Stress Rheometer (CSR Carri-Med model CSL 500, Dorking, Surrey, UK) with flow software. Gelatin solutions of different concentrations were analyzed as a function of temperature. Shear stress sweep of gelatin solution (10 mg/ml) has been carried out at 28°C, 40°C and 50° C. Gelatin solutions (15 mg/ml and 30 mg/ml) were analyzed at 25°C, 40°C, 50°C and 60°C. At a concentration of 30 mg/ml shear stress sweep has been carried out at 10°C. The sample was equilibrated for 5 min before the shearing experiment was started. The measuring geometry used was 4 cm cone and plate with truncation of 59 μm. The range of stress applied varied between 2 to 5 Pa depending on the angular velocity in the pre-shear experiment. The ascent and descent time were 2 min each. Shear stress sweep of the gelatin solutions were performed in triplicate and average values was taken for plotting. A flow curve was obtained by plotting log viscosity and log shear rate.

3.2.10. Expansion ratio of extruded products

Expansion ratio of the extruded products was determined as the ratio of the diameter of the extruded product to die diameter and expressed as percentage.

$$\text{Expansion ratio} = \frac{\text{Diameter of the extruded sample}}{\text{Die diameter}} \times 100$$

3.2.11. Water absorption capacity (WAC) of extruded products

Water absorption capacity of extruded products was determined by the method of Sousulski (1962). About 0.3 g of extruded sample was taken in a pre-weighed dried centrifugation tube. After weighing the sample with tube, 5 ml of water was added and mixed by using vortex mixer. The sample was kept for 30 minutes for the absorption of water and then centrifuged at 7000 x g for 10 minutes, so that excess water can get released from sample. The released water was decanted by inverting the tubes at an angle of 45° for 30 minutes at 50°C. The tubes were weighed again. The water absorption capacity was expressed as gram of water absorbed per gram of dried material. The average of three values was reported as WAC of dried material.

3.2.12. Color analysis

Color of the extruded products was determined using a Hunter Lab (Hunter Lab, Mini Scan XE Plus, Sunset Hills Road, Reston, Virginia, USA), color-measuring instrument. Extruded products were powdered using a mixer. The powder was then sieved through a sieve having mesh size of 250 μm. The particles passed through this mesh size were collected and allowed to sieve through another sieve with a mesh size of 150 µm. The particles, which are retained in this second sieve, are collected for color measurement. A uniform thick layer of the powder is taken in the sample holder of the colorimeter and values corresponding to L*, a* and b* were measured. Measurement has been carried out in triplicate and average value was taken for plotting.

3.2.13. Texture analysis

Crispness

Texture analysis of extruded products from different flours (rice, wheat and ragi) and fish mince (ribbonfish/Bull's eye fish) has been carried out using the Stable Microsystems TA-XT2 texture analyzer (Stable Microsystems, Godalming, UK). After a trigger force of 10g was attained the probe then proceeded to penetrate into the extruded product to a depth of 15mm. At this depth the maximum force reading (the resistance to penetration) was obtained and translated as the fracturibility of the extruded product.

Stable Microsystems TA XT2 texture analyzer setting.

- Mode : Measure Force in Compression
- Option : Return to start
- Pre-test speed : 1.0 mm/s
- Test speed : 2.0 mm/s
- Post test speed : 10.0 mm/s
- Distance : 15 mm
- Trigger type : Auto – 10g
- Data Acquisition rate : 500pps

5 mm diameter cylinder stainless probe (P5), Using 25Kg load cell

Breaking strength

The breaking strength of the extruded products was measured using Lloyd texture analyzer (Ametek, Lloyd instruments Ltd., Ametek Inc., Model LRX Plus, Hampshire, UK) attached with a Warner-Bratzler shear attachment. Instrument was set up to 'Compression to limit' mode, with a pre-load of 2 N and test speed of 500 mm/minute. The stiffness of the force required to break the product was measured. The measurement was carried out 10 times for each sample to get consistent results. The measurements were recorded in a computer with NEXYGEN, Ver. 4.1, software and breaking strength was expressed as Newton.

3.2.14. Determination of bloom strength of gelatin gels

Bloom value was determined using a TA-XT2 Texture analyzer (Stable Microsystems, Godalming, UK) according to the method described by Stable Micro System. Gelatin (5 g) was placed in cold water to make 5% w/v solution, stirred with a glass rod, covered and allowed to stand at room temperature (not more than 22^{o}C) for 3 hrs. After this time, the mixture was heated in a water bath at 60^{o}C (but not exceeding) and stirred on a magnetic stirrer for 15 min to dissolve the gelatin completely. Gelatin solution (150 ml) was immediately poured into standard bloom jar (SCHOTTGLAS. Mainz. Bloom test vessel. Product No. 2112501) over which a cover was placed. After 2 min bloom jars were kept in the cold room (4^{o}C) overnight (17 hrs), and immediately tested using Stable Microsystems TA-XT2 texture analyzer. The bloom jar was placed centrally under the standard probe and the penetration test was commenced. After a trigger force of 4 g was attained the probe proceeded to penetrate into the gel to a depth of 4mm. At this depth, the maximum force reading (the resistance to penetration) was obtained and translated as the Bloom strength (g) of the gel.

TA-XT2 Setting

- Mode : Measure force in compression
- Option : Return to start
- Pre-test speed : 0.5 mm/s
- Test speed : 0.5 mm/s
- Post test speed : 0.5 mm/s
- Distance : 4 mm
- Trigger type : Auto-4g
- Data Acquisition rate : 200 pps
- 0.5 Radius cylinder (P/0.5R) using 5 Kg load cell.

The method described corresponds to the British standard method for sampling and testing gelatins (BSI 757, 1975).

3.2.15. Sodium Dodecyl Sulphate – Poly Acrylamide Gel Electrophoresis

Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) of purified gelatin was carried out under reduced condition according to the method as described by Laemmli (1970). Electrophoresis was carried out using polyacrylamide gel slabs of 10x8 cm (length x width) in a vertical slab gel electrophoresis apparatus (Model SE 260; Hoefer Pharmacia Biotech Inc., USA). A discontinuous gel of acrylamide concentration T% = 10 and C% = 25 was used. For polymerization of the gel, TEMED (N, N, N', N'-tetramethylene diamine), used as the initiator and APS (ammonium persulphate) as catalyst. The gels were cast in a duel gel caster and thickness of the gel was 0.75 mm and the number of wells in each slab was 10.

One gram of gelatin was mixed with 3 ml of treatment buffer (Tris-HCl, pH 7.0, 10% SDS, 20% glycerol, 0.2% 2-mercaptoethanol and 0.02% bromophenol blue) and macerated. Then the samples were heated on a boiling water bath for two minutes, cooled and centrifuged at 8000 rpm for five minutes to get a clear supernatant. Supernatant was stored in vials and kept at –20^{o}C till electrophoresis was carried out.

Two microlitre of the clear solution was loaded into the wells of the gel. Run was carried out on a constant current mode using an electrophoresis power pack (Model PS-3000; Hoefer Pharmacia Biotech Inc., USA). A constant current of two milliampere per well of the gel was applied during the run. Run was terminated when the dye touched the bottom of gel. A standard sigma marker of wide range molecular weight was loaded into separate wells of the gel. After completing the run, gel was stained in comassie brilliant blue R-250 (0.025% in 40% methanol and 7% acetic acid) overnight. Gels were destained using acetic acid- methanol mixture (7% acetic acid and 2% methanol) repeatedly till protein bands were clearly visible. Molecular weight of the protein bands obtained in the sample was approximated by measuring the relative mobility of the standard molecular weight markers.

3.2.16. Setting index of gelatin

Different concentration of gelatin *viz.*, 10, 20, 30 and 40 mg/ml was prepared in test tube and kept at 5^{o}C for different durations. At periodic intervals visual observation was made for solidification process. The solidification process was given index depending on the extent of solidification. A maximum index of ‘100’ was taken as complete solid and ‘0’ as complete liquid. A plot of time in minutes and setting index was obtained.

3.2.17. Statistical analysis

The results obtained are the mean of three trials and the standard deviation has been found out. Two way analysis of variance technique was used in order to ascertain, whether there is any significant difference in the quality attributes of extruded products prepared with and without fish meat or between the samples prepared at different extrusion variables like barrel temperature, screw speed and die diameter.

IV. EXPERIMENTAL RESULTS

4.1. Composition of raw material used for extrusion

Ribbonfish (*Trichiurus spp.*) and bull's eye fish (*Priacanthus spp.*) can be classified under lean variety fish as the fat content was found to be less than 2%. The proximate composition of two fish used in the study is given in Table 2. The moisture content of both the fish were around 78%. The protein content of ribbonfish and bull's eye fish mince were 17.91% and 16.71% respectively. The proximate composition of rice flour, wheat semolina and ragi flour is given in Table 3. The data showed that the moisture content of rice flour, wheat semolina and ragi flour was 5.5%, 7.5% and 11.37% respectively. The protein content in the flours varied between 7-12% and fat content was less than 1%. Ash content of ragi was comparatively higher than rice and wheat semolina.

The amino acid composition of ribbonfish and bull's eye fish mince is given in Table 4. The amino acid profile revealed that both the fish were having a higher proportion of alanine, glutamic acid, lysine and leucine. The lysine content of ribbonfish and bull's eye fish meat was 10.57 and 9.79 (as percentage amino acids) respectively. Other major amino acids present in both the fish were aspartic acid, glycine and valine.

Fatty acid composition of fat extracted from ribbonfish and bull's eye fish is given in Table 5. The fatty acid profile revealed that the major fatty acid present in ribbonfish is erucic acid or cetoleic acid (C22 : 1n9) and that of bull's eye fish were heptadecanoic acid (C17 : 0) and arachidic acid (C20 : 0). The proportion of EPA and DHA were higher in both the fish analyzed. The sum of saturates in the fat extracted from ribbonfish and bull's eye fish accounts for 43.02% and 43.92% respectively. Polyenes accounted for 24.84% in ribbonfish and 17.56% in bull's eye fish.

4.2. Differential Scanning Calorimetry

The thermogram of the ribbonfish meat (Fig. 5 A) showed three transitions (Table 6). The first and second transitions were at T_m 33.17^oC and 48.85^oC and the third transition was at T_m 60.96^oC. The three transitions for bull's eye fish meat (Table 6, Fig. 5 B) were at 38.35^oC, 47.72^oC and 63.02^oC respectively. The enthalpy change value for ribbonfish

Table 2. Proximate composition of the fish mince used for extrusion process

Parameters	Ribbonfish	Bull's eye fish
Moisture (g/100 g meat)	78.01 (1.4)	78.8 (0.9)
Protein (N x 6.25) (g/100 g meat)	17.91 (0.19)	16.71 (0.36)
Fat (g/100 g meat)	1.18 (0.23)	1.28 (0.18)
Ash (g/100 g meat)	3.08 (0.24)	2.94 (0.48)

i) Values in parenthesis are standard deviation, n = 3

ii) All the values are on wet meat weight basis

Table 3. Proximate composition of different flours used in the study

Parameters	Rice flour	Wheat semolina	Ragi flour
Moisture (g/100 g meat)	5.5 (0.23)	7.5 (0.31)	11.37 (0.45)
Protein (N x 6.25) (g/100 g meat)	7.67 (0.10)	11.88 (0.16)	8.25 (0.09)
Fat (g/100 g meat)	0.31 (0.05)	0.45 (0.02)	0.28 (0.04)
Ash (g/100 g meat)	2.0 (0.14)	3.25 (0.49)	4.58 (0.53)

Values in parenthesis are standard deviation, n = 3

Table 4. Amino acid composition of two selected fish species

Amino acids	Ribbonfish (*Trichiurus spp.*) Mean ± S.D	Bull’s eye fish (*Priacanthus spp.*) Mean ± S.D
Asp	7.82 ± 0.25	7.96 ± 0.5
Glu	12.24 ± 0.6	11.84 ± 0.34
h. pro	0.37 ± 0.05	0.29 ± 0.1
Ser	4.49 ± 0.1	4.42 ± 0.08
Gly	7.22 ± 0.27	7.51 ± 0.34
His	0.34 ± 0.008	0.45 ± 0.05
Arg	5.25 ± 0.01	5.79 ± 0.02
Thr	5.26 ± 0.02	5.14 ± 0.16
Ala	13.05 ± 0.196	13.04 ± 0.41
Pro	1.39 ± 0.05	1.8 ± 0.46
Tyr	3.43 ± 0.07	3.37 ± 0.07
Val	6.22 ± 0.15	6.48 ± 0.2
Met	1.99 ± 0.03	1.96 ± 0.54
Cys	0.46 ± 0.005	0.53 ± 0.06
I leu	4.81 ± 0.05	4.63 ± 0.02
Leu	8.94 ± 0.11	8.7 ± 0.1
Phe	3.37 ± 0.1	3.3 ± 0.03
Trp	2.77 ± 0.13	2.97 ± 0.45
Lys	10.57 ± 0.17	9.79 ± 0.76

% Amino acids in fish (S.D – standard deviation)

Table 5. Fatty acid composition of ribbonfish (*Trichiurus spp.*) and bull's eye fish (*Priacanthus spp.*)

Fatty acids	Ribbonfish Mean ± S.D	Bull's eye fish Mean ± S.D
C 14:0 (Myristic)	5.433 ± 1.22	2.384 ± 0.13
C 16:0 (Palmitic)	5.725 ± 1.43	6.597 ± 1.44
C 16:1 (Palmitoleic)	7.506 ± 2.77	6.751 ± 1.66
C 17:0 (Heptadecanoic)	13.005 ± 4.31	12.763 ± 3.65
C 18:0 (Stearic)	8.575 ± 1.54	5.156 ± 1.68
C 18:1n9c (Oleic)	5.186 ± 1.11	7.120 ± 1.87
C 18:2n6c (Linoleic)	4.331 ± 1.98	9.599 ± 2.70
C 18:3n3 (Linolenic)	5.146 ± 1.49	1.617 ± 0.09
C 20:0 (Arachidic)	6.176 ± 2.22	12.671 ± 1.55
C 20:1n9 (cis-11-Eicosenoic)	ND	ND
C 20:3n3 (cis-11, 14, 17-Eicosatrienoic)	ND	ND
C 20:5n3 EPA	11.839 ± 1.65	6.989 ± 1.54
C 22:0 (Behenic)	10.151 ± 3.76	9.838 ± 1.59
C 22:1n9 Erucic acid/Cetoleic	16.140 ± 4.44	9.557 ± 2.67
C 22:6n3 DHA	7.857 ± 1.79	8.958 ± 2.55
Sum of saturates	43.028 ± 3.87	43.919 ± 3.22
Sum of monoenes	39.201 ± 2.69	38.517 ± 2.98
Sum of polyenes	24.842 ± 2.98	17.564 ± 1.99

% (14 Fatty acids) (S.D-standard deviation; ND- Not determined)

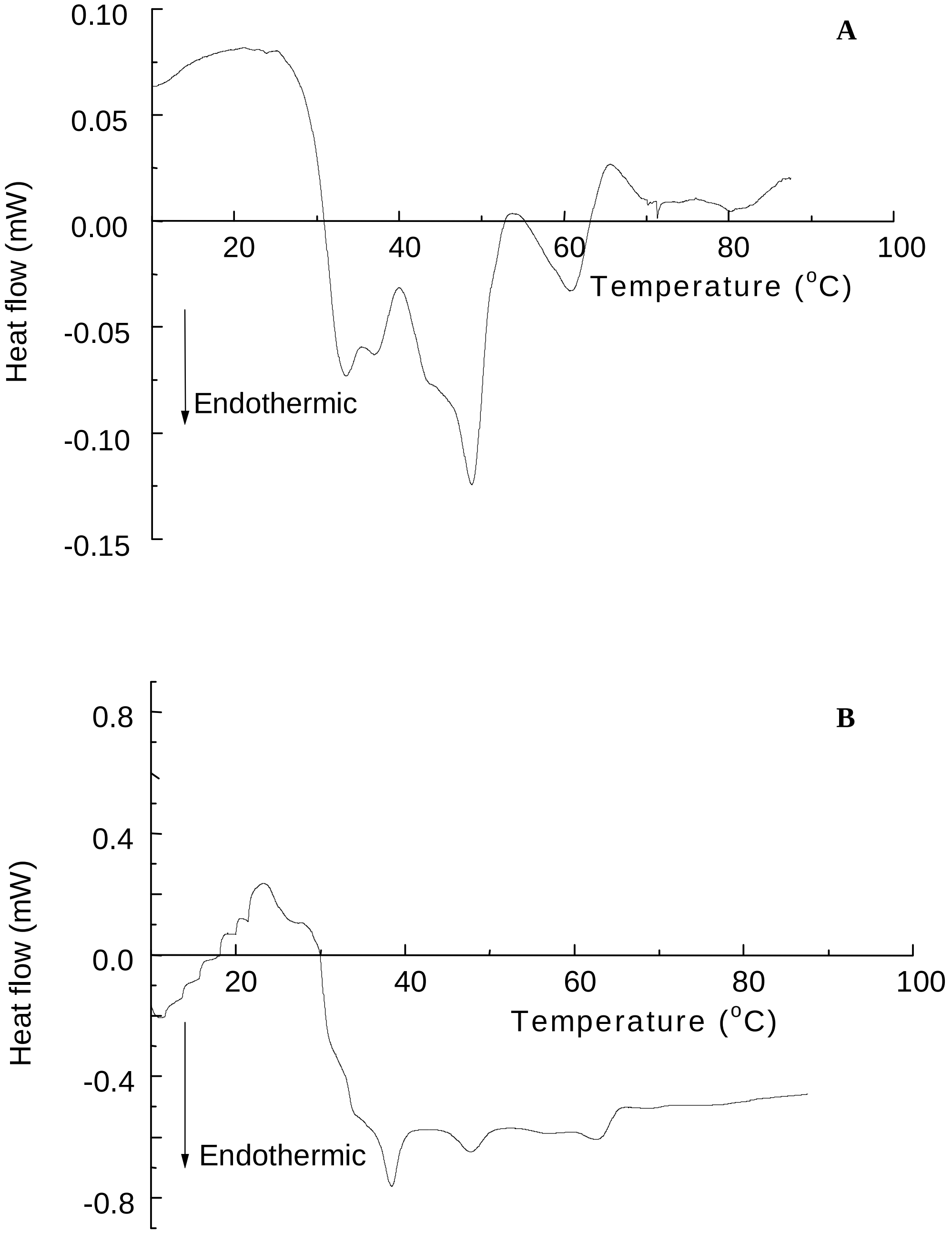

Fig. 5: DSC profile of A: Ribbonfish meat; B: Bull's eye fish meat. The experiment was carried out at a heating rate of 0.5°C/min.

Table 6. Onset and peak gelatinization/denaturation temperatures and enthalpy values of rice flour, wheat semolina, ragi flour and fish meat

Flours/Fish	Onset point (^oC)	Peak T_m (^oC)	Enthalpy (J/g)
Rice	58.69	64.73	0.2476
Wheat	49.13	58.16	0.5788
Ragi	65.92	69.72	0.4475
Ribbonfish meat	29.84	33.17	0.2038
	44.09	48.85	0.2582
	54.94	60.96	0.1053
Bull's eye fish meat	33.13	38.35	0.3252
	45.25	47.72	0.0791
	60.27	63.02	0.0664

and bull’s eye fish meat due to denaturation of myosin was 0.2038 J/g and 0.3252 J/g respectively.

The onset and peak gelatinization temperature and the enthalpy value of rice flour, wheat semolina and ragi flour solutions (10% w/v) are given in Table 6 and the thermograms are given in Fig 6A, B & 7. The peak value for the endothermic transition was lower for wheat semolina (58.16^{o}C) and higher for ragi flour (69.72^{o}C). The enthalpy values for rice and ragi flours were 0.2476 J/g and 0.4475 J/g respectively. Among the flours/semolina the enthalpy value was maximum for wheat semolina (0.5788 J/g).

4.3. FT-Raman spectroscopy

Raman spectra of ribbonfish and bull’s eye fish meat are given in Fig. 8&9 and the intensity of the bands are given in Table 7&8. The aromatic amino acid phenylalanine showed a strong band at 1005 cm^{-1}. As the intensity of this band is not affected by the microenvironment or external factors the intensity of this band is used as internal standard for normalization. Two peaks at 760 and 1554 cm^{-1} are due to the tryptophan residue from the indole ring vibration. Among the several bands of the tyrosine, the tyrosine doublet band, which is located at 830 and 855 cm^{-1} are very useful for monitoring the protein conformation. The ratio of the intensity of bands I_{855}/I_{830} for ribbonfish was 0.64 and that of bull’s eye was 0.80. The bands assigned for Amide I was centered around 1660 cm^{-1}.

Raman spectra of rice flour, wheat semolina and ragi flour have been carried out both in the native and gelatinized state (Fig. 10-15 & Table 9-10). In the spectra of rice flour, there was only marginal change in intensity of bands after gelatinization, but the band at 1633 cm^{-1} and 3213 cm^{-1} increased. In the spectra of wheat semolina there was two additional peaks between 1663-1630 cm^{-1} and another between 1595-1528 cm^{-1}, which corresponds to Amide I and Amide II respectively. In wheat semolina and ragi flour after gelatinization the intensity of all bands decreased except the bands at 1633 cm^{-1} and 3213 cm^{-1}, which were increased. The characteristic of the Raman spectra of all polysaccharides with α (1-4) bonds between elementary links showed an intense band at 480 cm^{-1}. The intensity of CH_2 deformation in rice flour, wheat semolina and ragi flour was 0.47, 1.6 and 0.57 respectively.

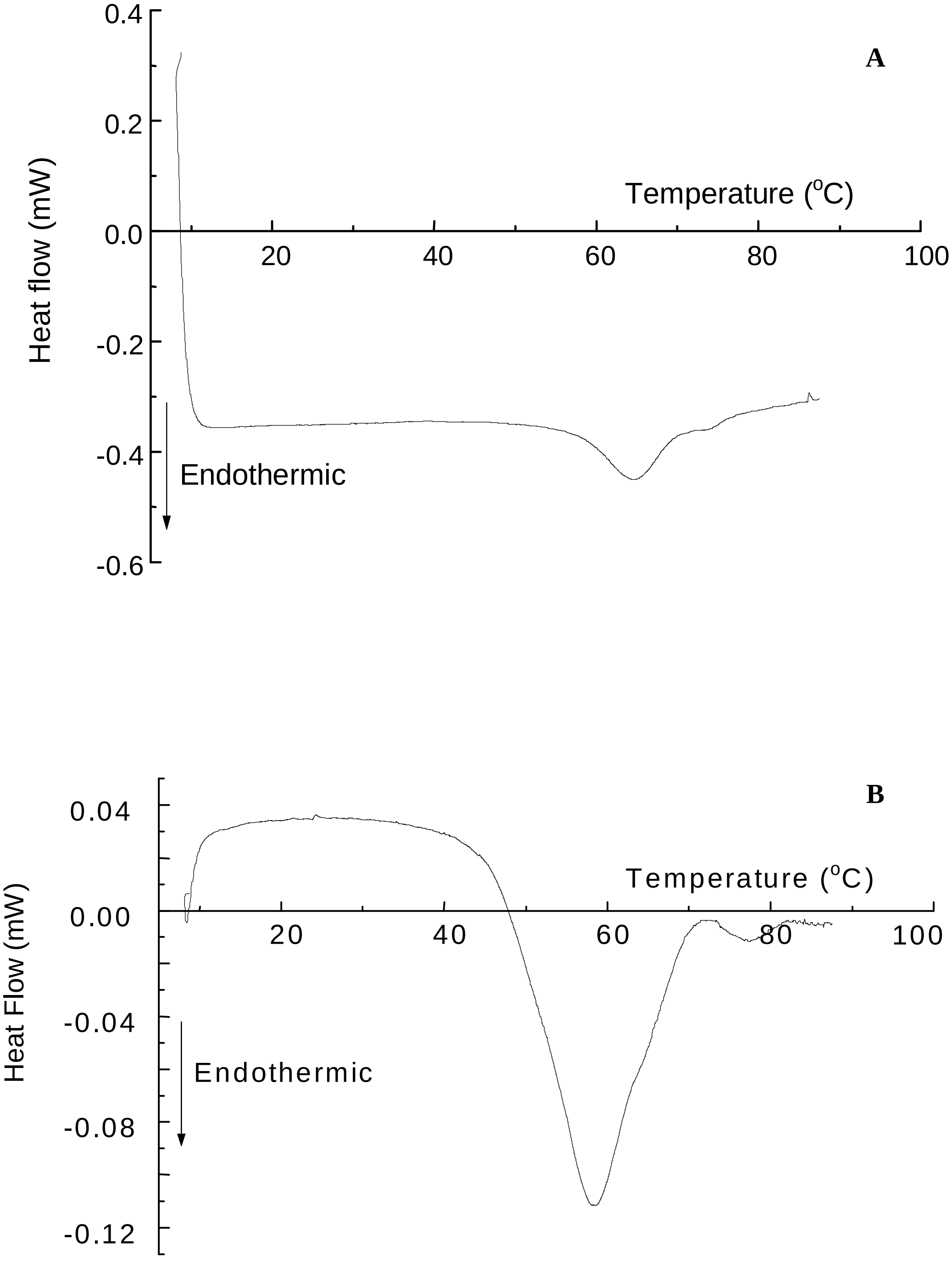

Fig. 6: DSC profile of A: Rice flour (10% w/v); B: wheat semolina (10% w/v) solution. (The solution was equilibrated at ambient temp for 15 minutes and the heating rate applied was 0.5^{o}C/min)

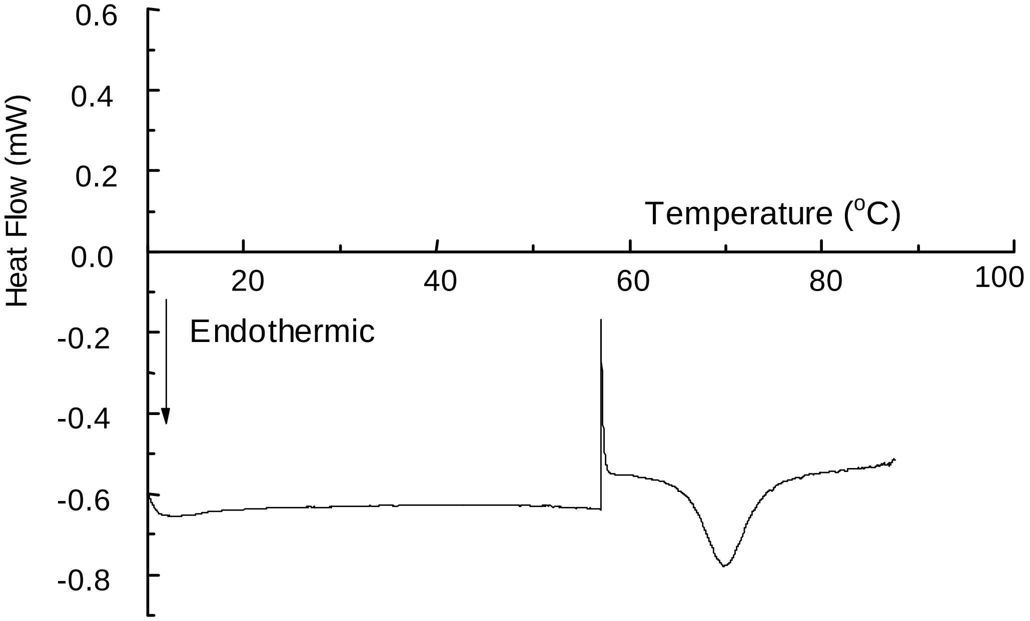

Fig. 7: DSC profile of ragi flour (10% w/v) solution. (The solution was equilibrated at ambient temp for 15 minutes and the heating rate applied was 0.5°C/min)

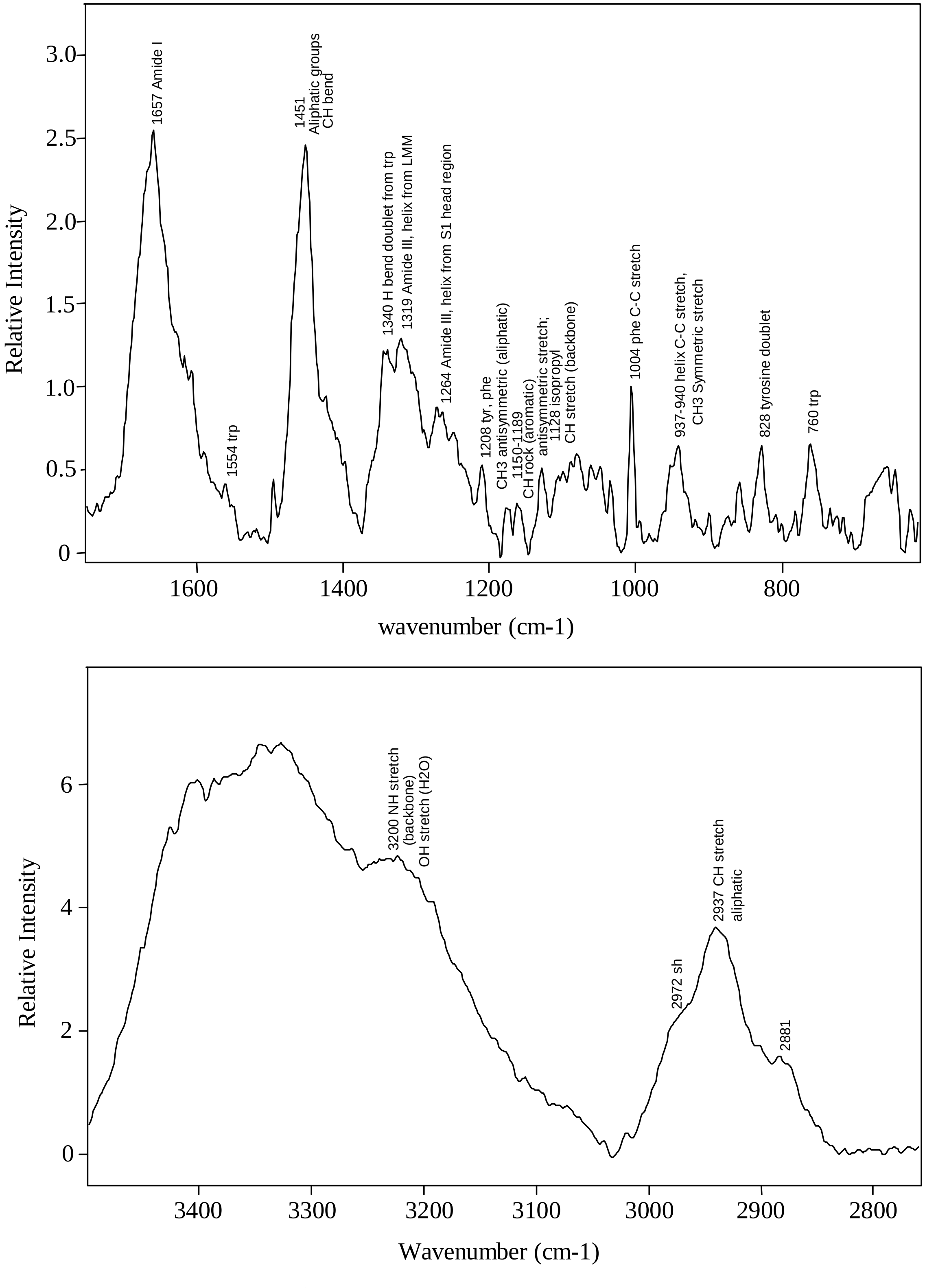

Fig. 8: FT-Raman spectra of ribbonfish meat. The spectra represent average of 128 scans.

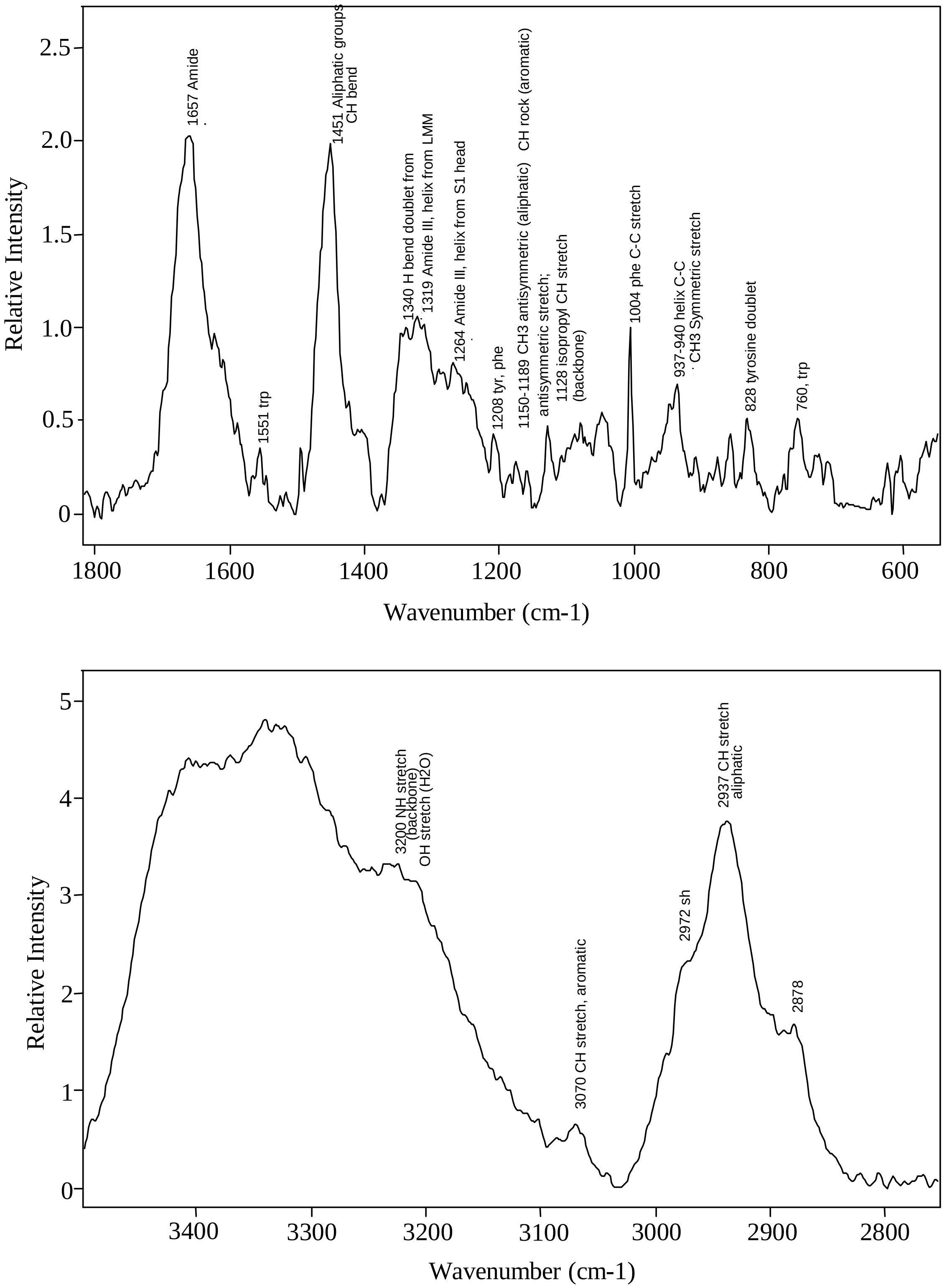

Fig. 9: FT-Raman spectra of bull's eye fish meat. The spectra represent average of 128 scans.

Table 7. FT- Raman spectra of fish meat (ribbonfish) - Relative peak intensity* and peak assignment

Wavenumber ± 2 cm^{-1}	Relative intensity
Trp (760)	0.577 ± 0.07
Tyr (830)	0.616 ± 0.03
Tyr (855)	0.396 ± 0.02
Helix C-C stretch, CH_3 symmetric stretch (937)	0.573 ± 0.09
β-sheet type structure (990)	0.181 ± 0.02
Phe, ring band (1034)	0.409 ± 0.05
Isopropyl anti symmetric stretch CH stretch back bone (1128)	0.488 ± 0.02
CH_3 antisymmetric (aliphatic) CH_3 rock (aromatic) (1160)	0.252 ± 0.04
β-sheet type (1239)	0.509 ± 0.08
Amide III (random coil) (1245)	0.657 ± 0.06
Amide III (1264)	0.807 ± 0.10
Amide I (1320)	1.210 ± 0.08
H band doublet from trp (1340)	1.093 ± 0.11
(sh*, residue vibration) asp, glu, lys (1425)	0.908 ± 0.02
Aliphatic groups, CH bend (1451)	2.316 ± 0.20
Trp (1554)	0.343 ± 0.05
Amide I (1660)	2.432 ± 0.28
CH stretch, aliphatic (2940)	3.536 ± 0.31
Shoulder (2888)	1.495 ± 0.16
Shoulder (2976)	2.148 ± 0.16
Shoulder (2969)	2.303 ± 0.15

*Mean ± standard deviation

Table 8. FT- Raman spectra of fish meat (bull's eye fish) - Relative peak intensity* and peak assignment

Wavenumber ± 2 cm^{-1}	Relative intensity
Trp (760)	0.563 ± 0.09
Tyr (830)	0.515 ± 0.02
Tyr (855)	0.413 ± 0.04
Helix C-C stretch, CH_3 symmetric stretch (937)	0.548 ± 0.13
B-sheet type structure (990)	0.177 ± 0.06
Phe, ring band (1034)	0.385 ± 0.11
Isopropyl anti symmetric stretch CH stretch back bone (1128)	0.438 ± 0.04
CH_3 antisymmetric (aliphatic) CH_3 rock (aromatic) (1160)	0.199 ± 0.04
B-sheet type (1239)	0.650 ± 0.07
Amide III (random coil) (1245)	0.704 ± 0.02
Amide III (1264)	0.788 ± 0.05
Amide I (1320)	1.050 ± 0.01
H band doublet from trp (1340)	1.006 ± 0.04
(sh*, residue vibration) asp, glu, lys (1425)	0.674 ± 0.07
Aliphatic groups, CH bend (1451)	1.938 ± 0.11
Trp (1554)	0.294 ± 0.06
Amide I (1660)	1.945 ± 0.11
CH stretch, aliphatic (2940)	3.482 ± 0.28
Shoulder (2888)	1.430 ± 0.16
Shoulder (2969)	2.199 ± 0.13

*Mean ± standard deviation

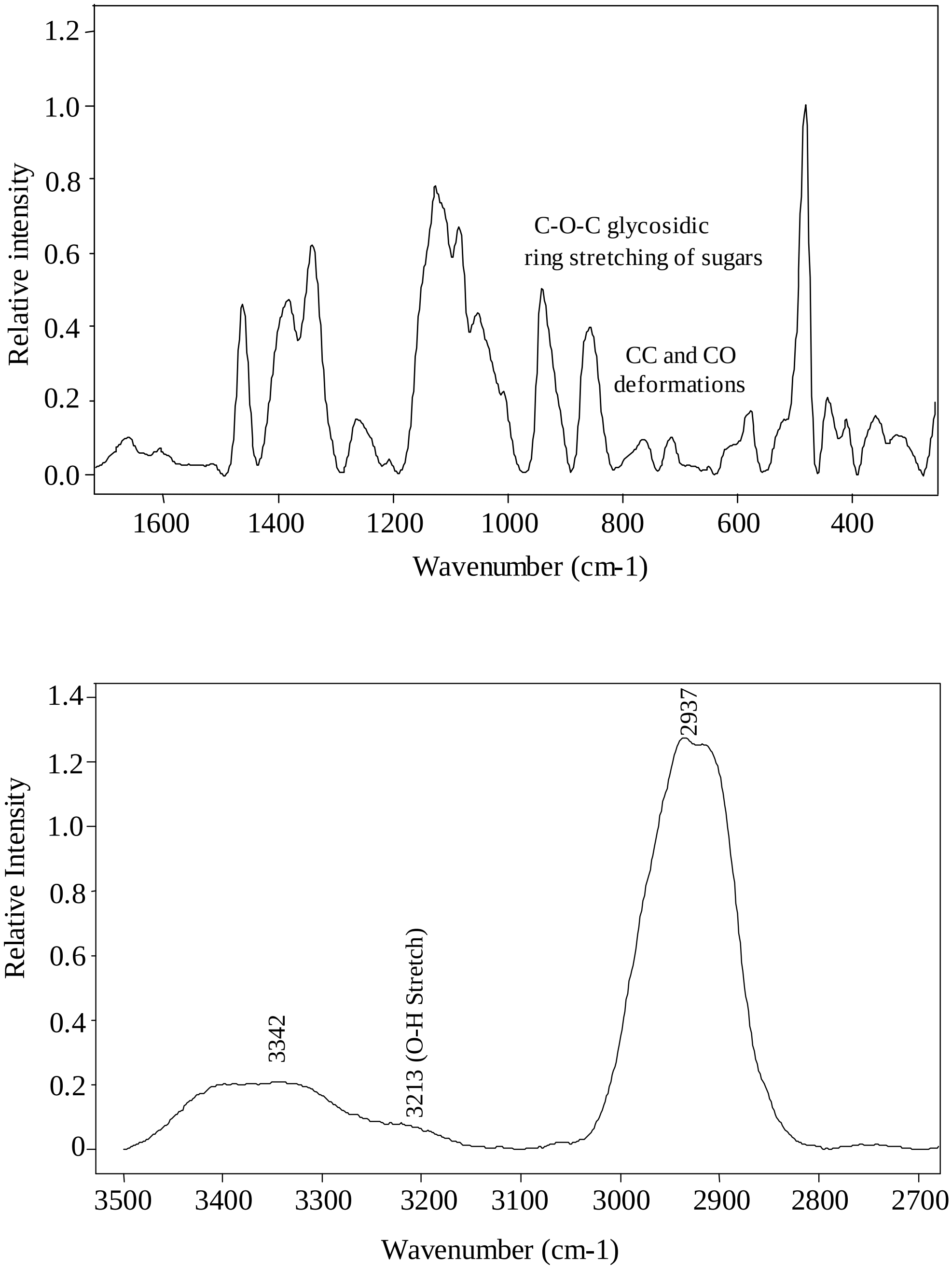

Fig. 10: FT-Raman spectra of native rice flour. Flour as such was used for the scan. The spectra represent average of 64 scans.

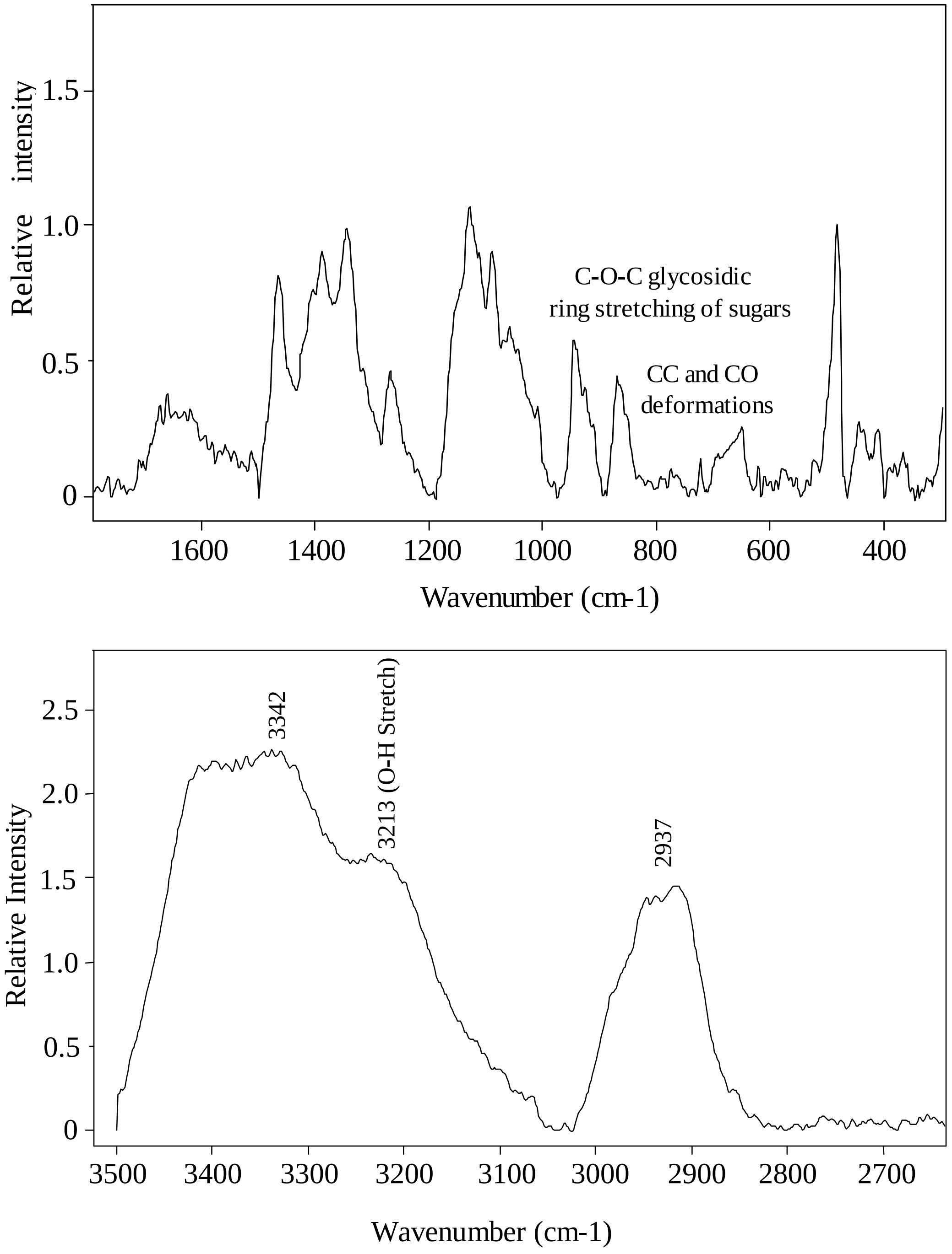

Fig 11. FT-Raman spectra of gelatinized rice flour. Gelatinization was carried out at 80°C for 30 minutes. The spectra represent average of 128 scans.

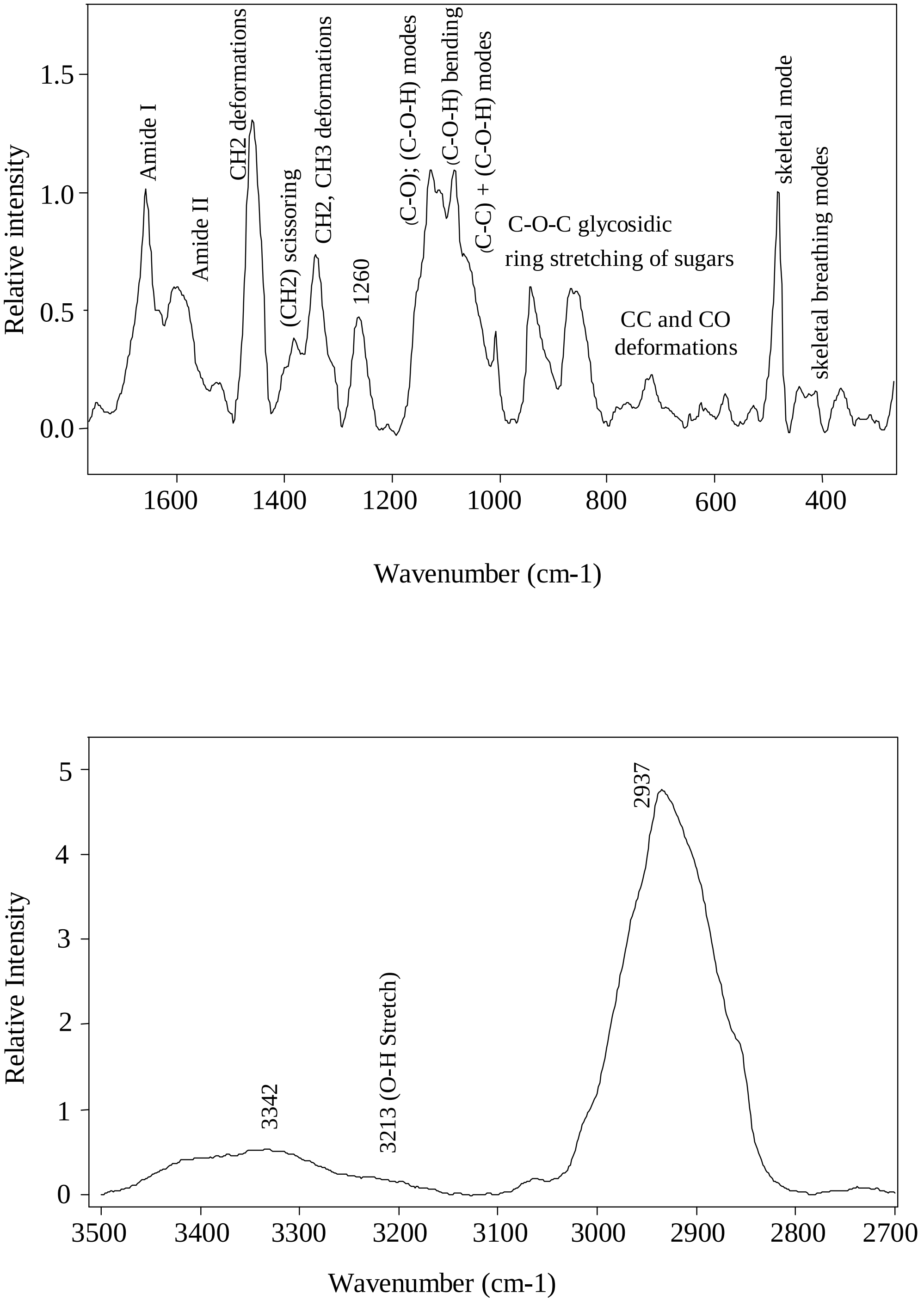

Fig. 12: FT-Raman spectra of native wheat semolina. Semolina as such was used for the scan. The spectra represent average of 64 scans.

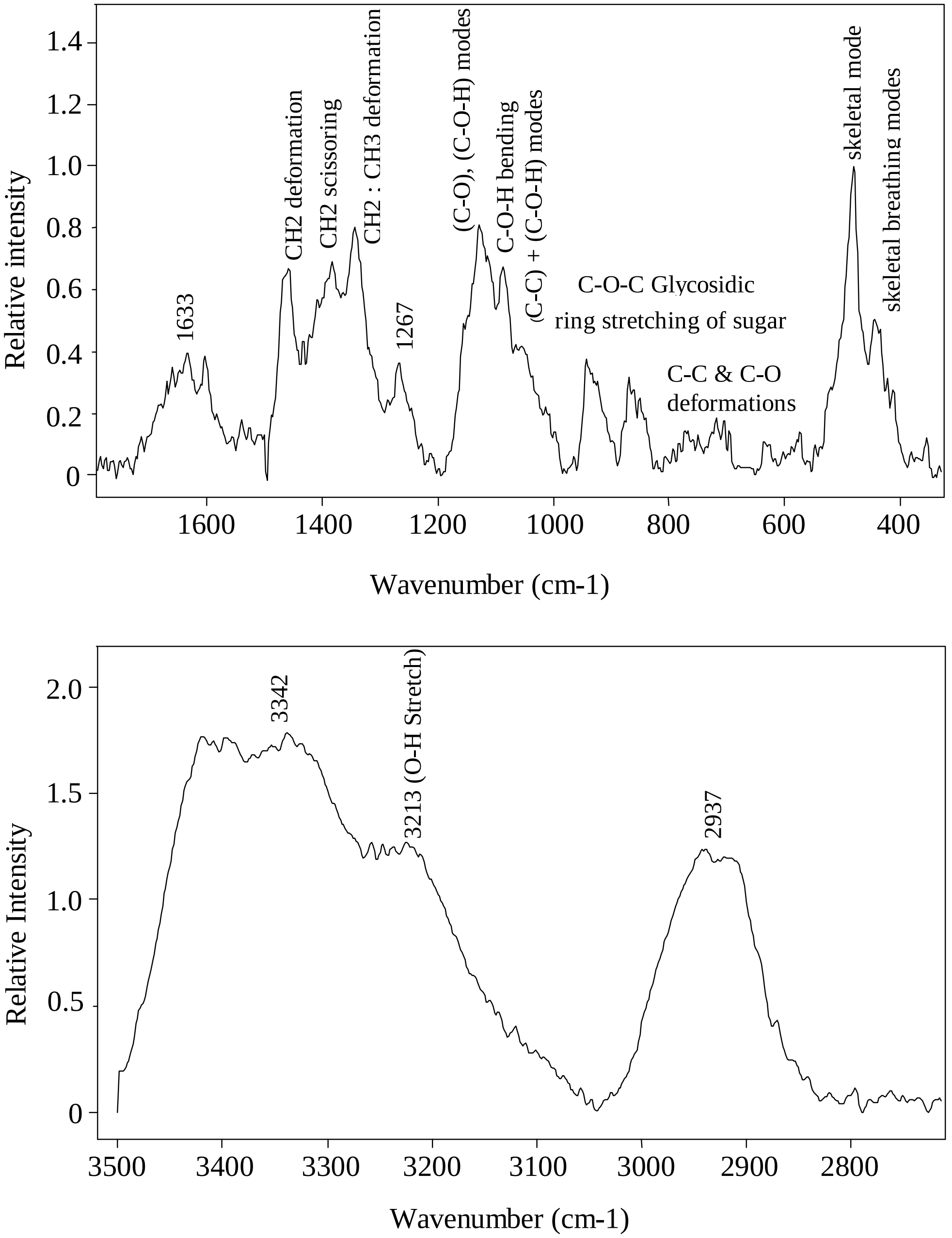

Fig. 13: FT-Raman spectra of gelatinized wheat semolina. Gelatinization was carried out at 80^{o}C for 30 minutes. The spectra represent average of 128 scans.

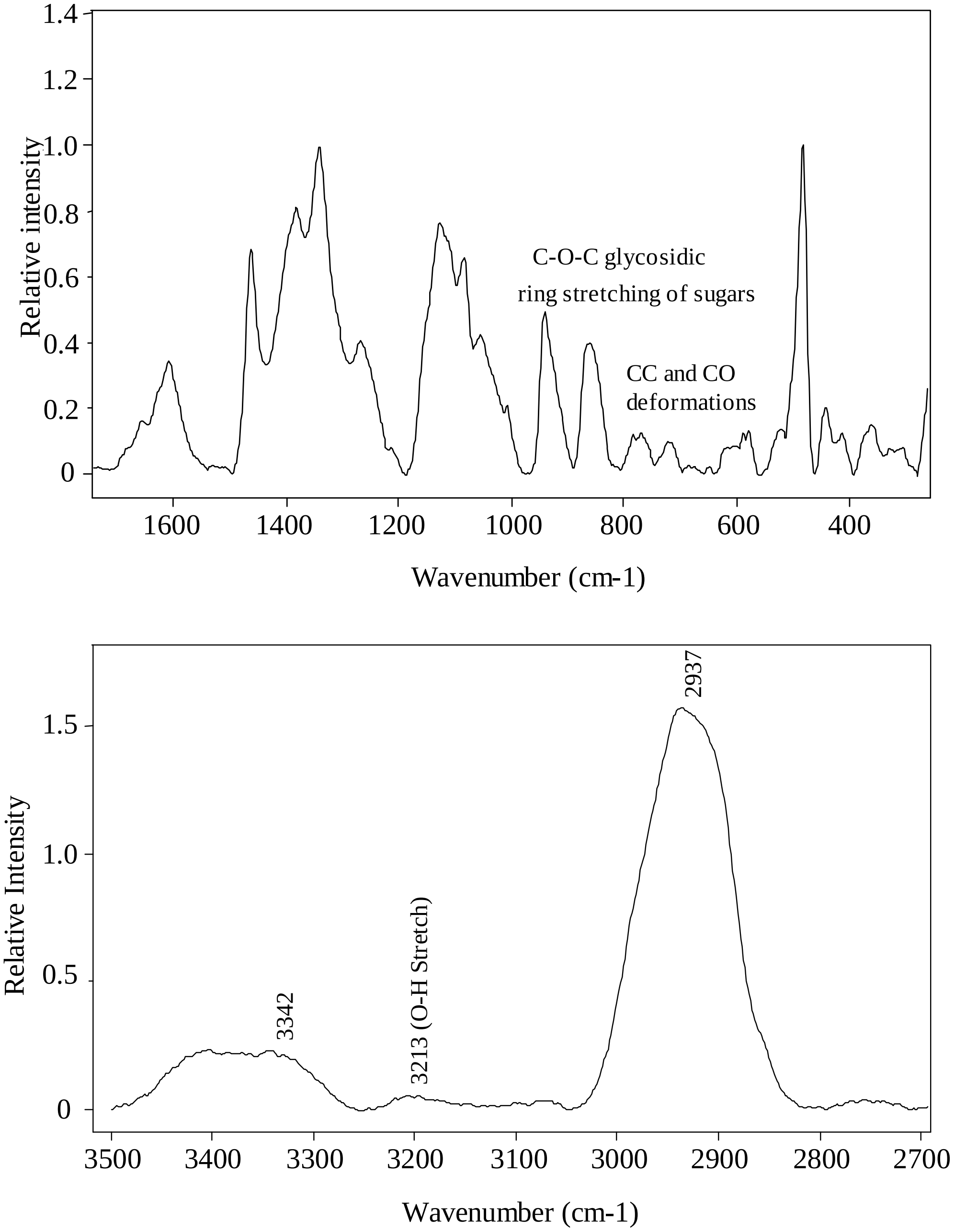

Fig. 14: FT-Raman spectra of native ragi flour. Flour as such was used for the scan. The spectra represent average of 64 scans.

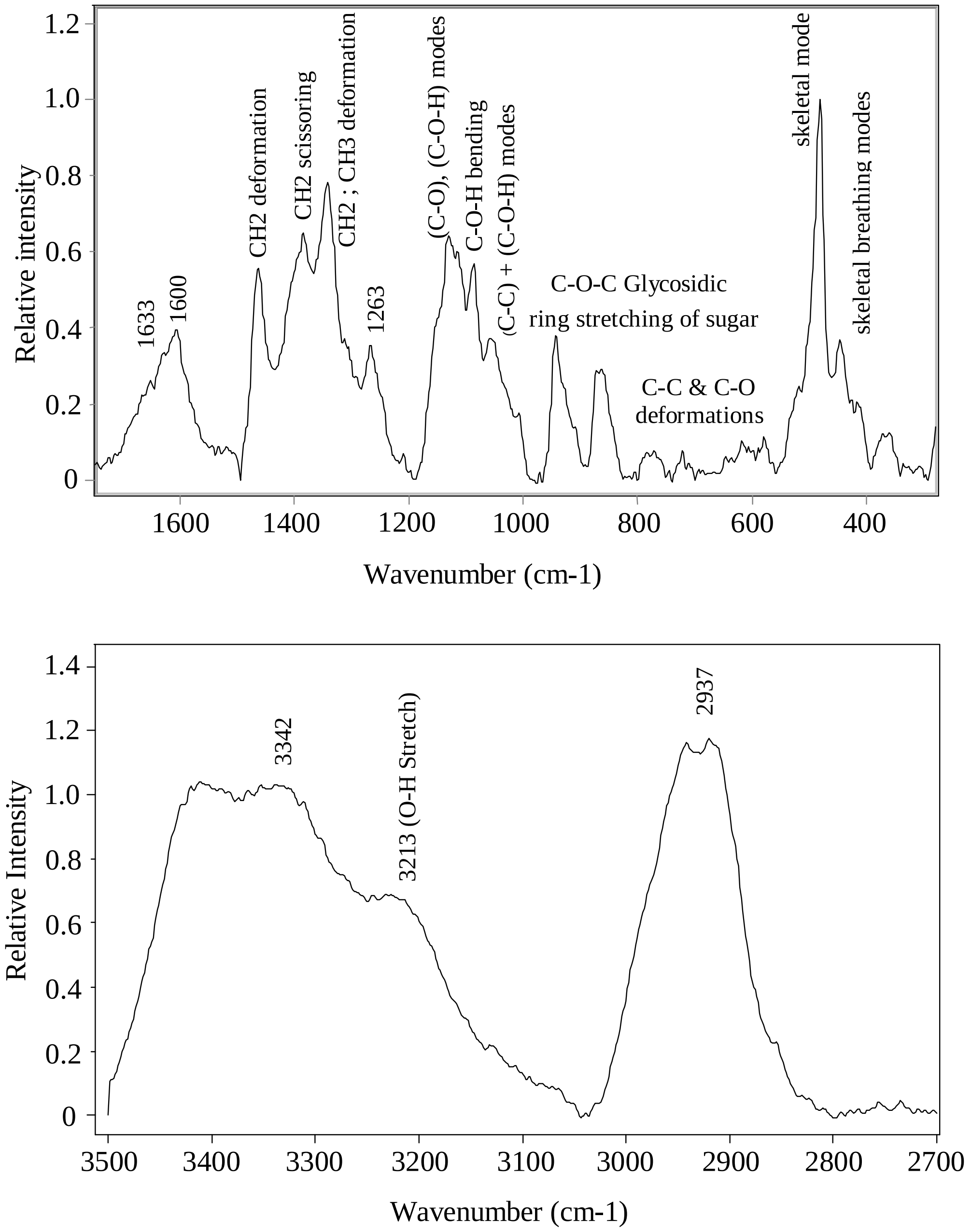

Fig. 15: FT-Raman spectra of gelatinized ragi flour. Gelatinization was carried out at 80^{o}C for 30 minutes. The spectra represent average of 128 scans.

Table 9. FT-Raman spectra: Relative intensity* of important peaks in different flours (native)

Wavenumbers	Rice	Wheat	Ragi
3332	--	0.637 ± 0.09	--
3062	--	0.248 ± 0.05	--
2936	1.269 ± 0.07	5.855 ± 0.96	1.443 ± 0.12
2185	0.049 ± 0.00	0.134 ± 0.01	0.145 ± 0.01
2074	0.045 ± 0.01	0.139 ± 0.03	0.131 ± 0.02
2059	0.045 ± 0.00	0.133 ± 0.04	0.138 ± 0.02
1656	0.104 ± 0.01	1.362 ± 0.31	0.197 ± 0.04
1633	--	0.627 ± 0.11	--
1606	0.086 ± 0.01	--	0.404 ± 0.06
1462	0.476 ± 0.03	--	0.577 ± 0.09
1459	--	1.606 ± 0.26	--
1380	0.502 ± 0.03	0.340 ± 0.04	0.608 ± 0.18
1340	0.650 ± 0.03	0.746 ± 0.01	0.768 ± 0.20
1266	0.163 ± 0.02	--	0.244 ± 0.14
1258	--	0.600 ± 0.11	--
1127	0.788 ± 0.03	1.239 ± 0.12	0.779 ± 0.02
1082	0.673 ± 0.03	1.306 ± 0.18	0.668 ± 0.01
1054	0.442 ± 0.02	--	0.433 ± 0.01
1005	0.222 ± 0.01	0.447 ± 0.04	0.217 ± 0.01
940	0.509 ± 0.02	0.520 ± 0.07	0.483 ± 0.01
860	0.389 ± 0.02	--	0.401 ± 0.01
855	--	0.582 ± 0.02	--
716	0.097 ± 0.01	0.253 ± 0.02	--
578	0.173 ± 0.01	0.133 ± 0.02	0.147 ± 0.02
480	1.00 ± 0.00	1.001 ± 0.00	1.00 ± 0.00
442	0.203 ± 0.01	0.205 ± 0.02	0.196 ± 0.02
426	--	0.188 ± 0.04	--
411	0.132 ± 0.02	0.212 ± 0.04	0.132 ± 0.01

*Mean ± standard deviation; (-- denotes no conspicuous peak)

Table 10. FT-Raman spectra: Relative intensity* of important peaks in different flour samples in gelatinized state

Wavenumbers (cm^{-1})	Rice	Wheat	Ragi
420	0.198 ± 0.04	0.366 ± 0.10	0.208 ± 0.00
480	1.002 ± 0.00	1.000 ± 0.00	1.000 ± 0.00
519	0.172 ± 0.05	0.321 ± 0.05	0.222 ± 0.02
735	0.026 ± 0.01	0.065 ± 0.03	0.034 ± 0.02
910	0.224 ± 0.04	0.188 ± 0.02	0.149 ± 0.02
940	0.535 ± 0.05	0.360 ± 0.02	0.400 ± 0.02
1057	0.567 ± 0.05	0.419 ± 0.02	0.387 ± 0.01
1082	0.812 ± 0.08	0.624 ± 0.05	0.589 ± 0.02
1128	0.972 ± 0.08	0.753 ± 0.05	0.684 ± 0.04
1380	0.880 ± 0.04	0.646 ± 0.05	0.662 ± 0.01
1633	0.348 ± 0.06	0.371 ± 0.02	0.326 ± 0.01
2908	1.418 ± 0.09	1.098 ± 0.08	1.178 ± 0.03
3213	1.529 ± 0.10	1.087 ± 0.12	0.663 ± 0.01
Other major peaks			
3328	2.175 ± 0.16	1.601 ± 0.16	1.039 ± 0.02
3233	1.577 ± 0.14	--	--
2947	1.352 ± 0.10	--	--
2937	1.370 ± 0.07	1.127 ± 0.10	1.169 ± 0.02
1461	0.817 ± 0.06	0.653 ± 0.02	0.566 ± 0.01
1341	0.961 ± 0.07	0.721 ± 0.07	0.787 ± 0.01
1266	0.424 ± 0.03	0.350 ± 0.01	0.364 ± 0.01
1005	0.301 ± 0.02	0.195 ± 0.01	0.188 ± 0.01
866	0.419 ± 0.06	0.295 ± 0.03	0.308 ± 0.02
442	0.274 ± 0.02	0.526 ± 0.02	0.339 ± 0.03

*Mean ± standard deviation; (-- denotes no conspicuous peak)

4.4. Small deformation testing of flours/semolina solutions

Small deformation test of 10% (w/v) solution of rice flour, wheat semolina and ragi flour has been carried out (Fig. 16&17). For all the flour samples G’ and G’’ value increased with an increase in temperature. Crossover points of samples are given in Table 11. The maximum rate of increase in G’ was between 60°C to 70°C. Since G’ increase faster than G’’, ‘crossover’ (which is the gel point), took place at a temperature of around 50-60°C. The crossover point of rice flour was at 61.63°C whereas that of wheat semolina and ragi flours was at 50.23°C and 52.68°C respectively. The maximum value of G’ for rice flour, wheat semolina and ragi flour were 6029 Pa, 118 Pa and 544 Pa respectively.

4.5. Phase contrast microscopy

Phase contrast micrograph of rice flour, wheat semolina and ragi flour in dry, wet and gelatinized state is given in Fig. 18-20. The micrographs showed swollen starch granules after gelatinization. The unheated flour solutions also showed swelling to some extent, which was found to be reversible (Fig. 18 B, 19 B, 20 B).

4.6. Protein-polysaccharide interaction

The protein-polysaccharide interaction was carried out with the protein extracted from ribbonfish meat and flours/semolina. The concentration of the proteins used for interaction studies was 10-10.5 mg/ml and flour/wheat semolina at 20% (w/v) in protein solution.

Dynamic viscoelastic behaviour of rice flour showed decreasing trend in G’ at 60°C and 80°C with time period (Fig. 21). However, at 70°C storage modulus (G’) increased with time period. The maximum G’ (102.6 KPa) was observed in the time sweep at 70°C. Tan δ values decreased in all the cases. With the addition of protein solution from ribbonfish meat G’ values decreased. Tan δ values also showed an increasing trend.

Time sweep of wheat semolina solution (20% w/v) revealed that G’ value increased with increase in time period irrespective of the temperature (Fig. 22). The maximum G’ value (8.25 KPa) was observed at a temperature of 70°C. Tan δ values showed a decreasing trend. Addition of protein solution from ribbonfish meat enhanced the G’ value irrespective of the temperature. With the addition of protein solution, the maximum G’

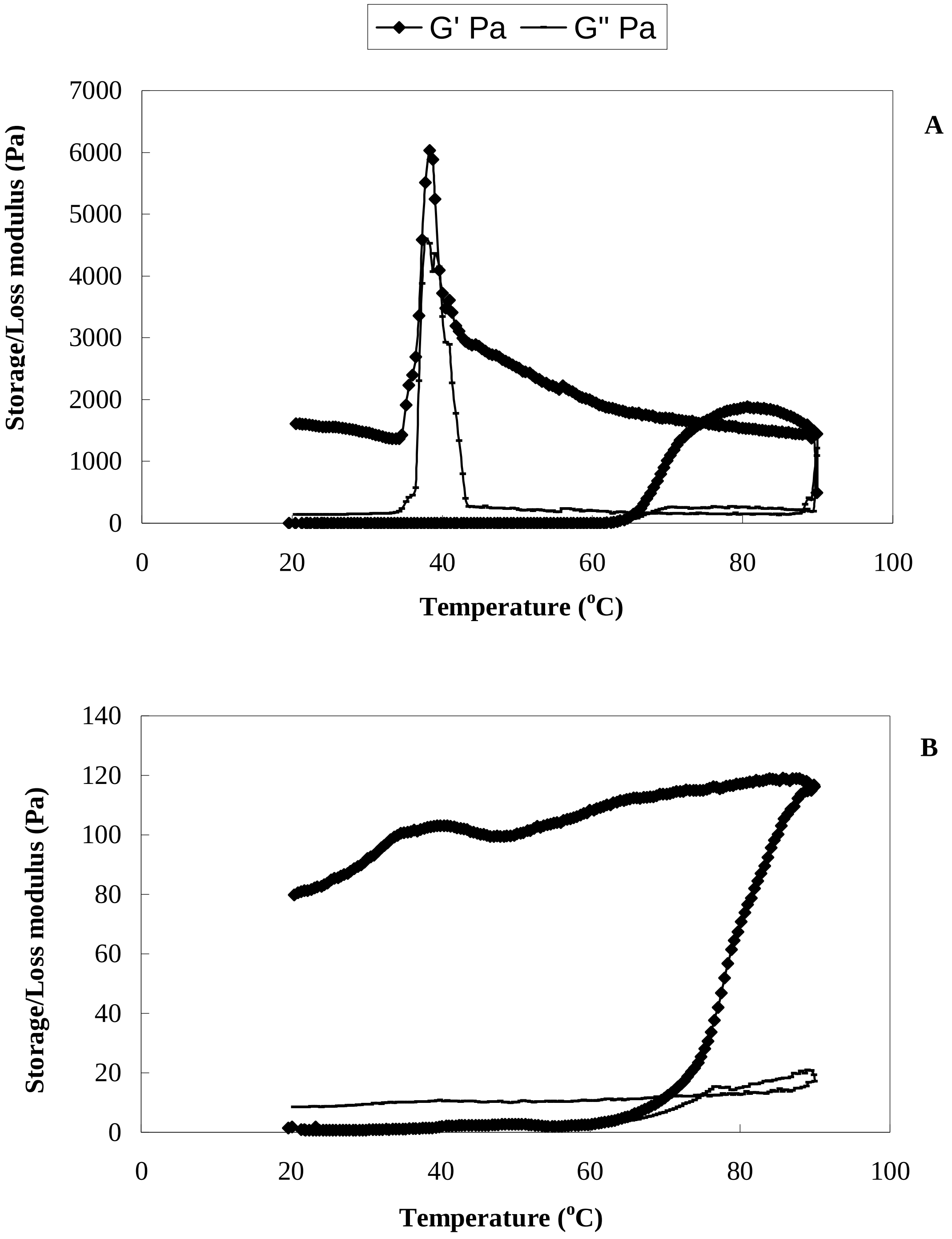

Fig. 16: Temperature sweep of A: 10% rice flour solution; B: 10% wheat semolina solution. The gap between geometry and peltier plate was 0.3 mm. Heating rate was 2°C/min.

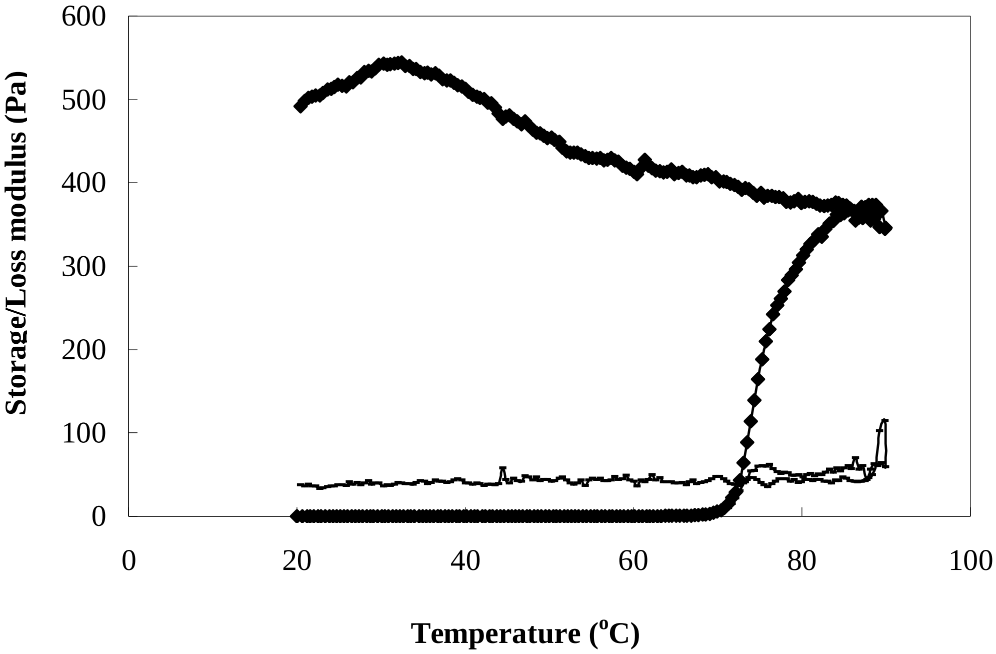

Fig. 17: Temperature sweep of 10% ragi flour solution. The gap between geometry and peltier plate was 0.3 mm. Heating rate was 2^oC/min.

Table 11. Cross over temperature obtained from dynamic rheological test for different flour solutions

Flours	Temperature (^{o}C)
Rice	61.63
Wheat	50.23
Ragi	52.68

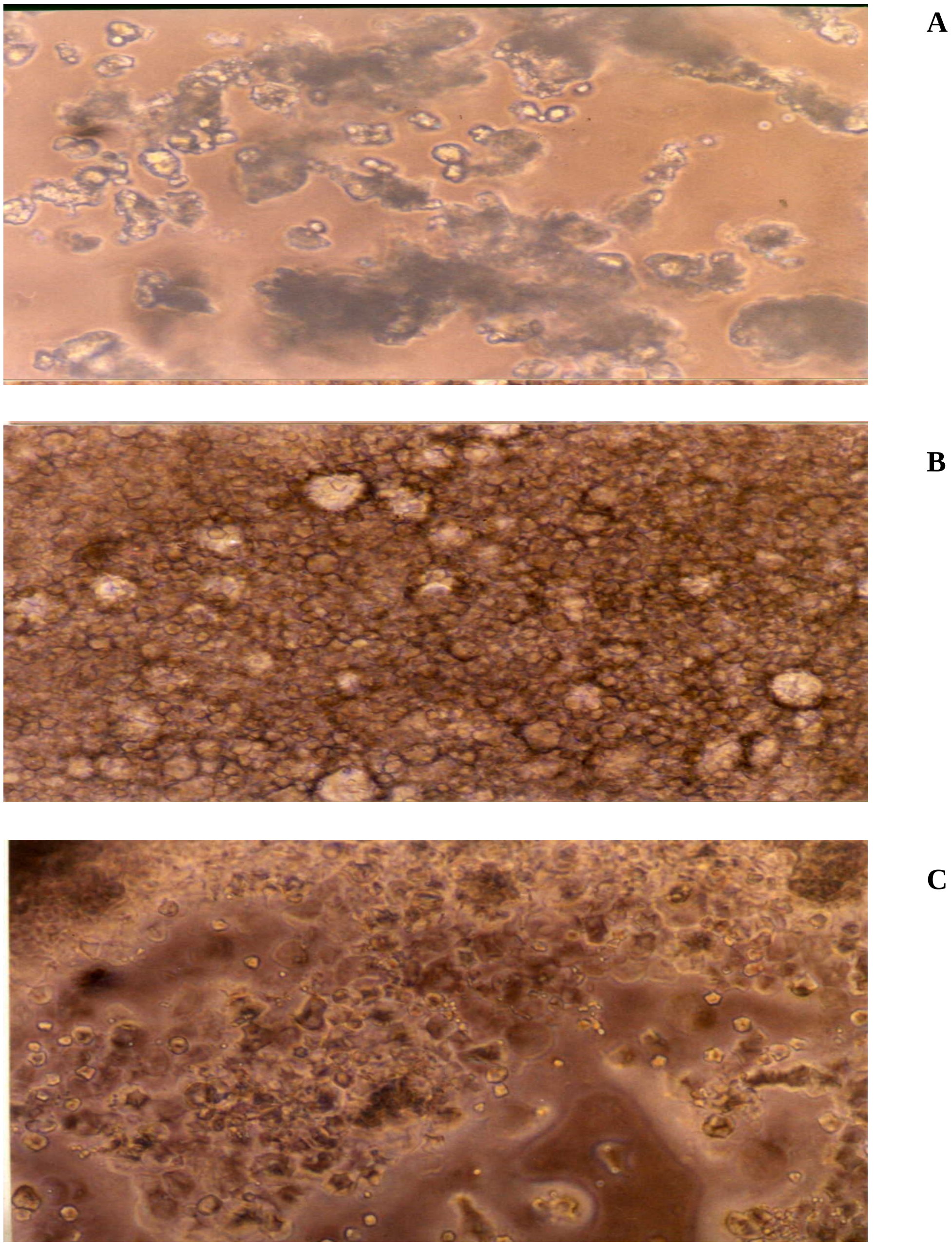

Fig. 18: Phase contrast microscopy of rice flour
A: Dry powder; B: Paste; C: Gelatinized
(Gelatinization was carried at 80°C for 30 minutes; Magnification- 400 x)

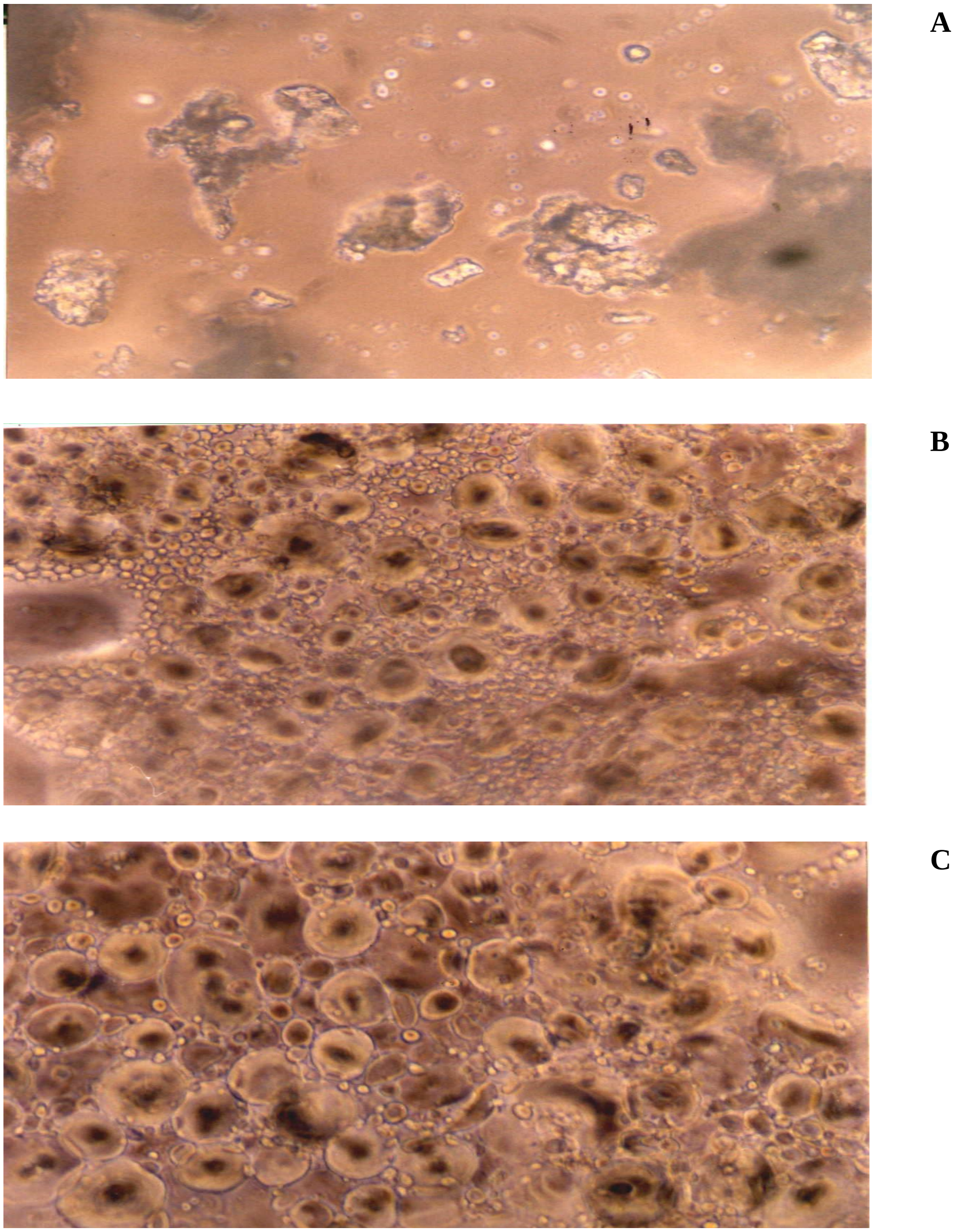

Fig. 19: Phase contrast microscopy of wheat semolina;
A: Dry powder; B: Paste; C: Gelatinized
(Gelatinization was carried at 80°C for 30 minutes; Magnification- 400 x)

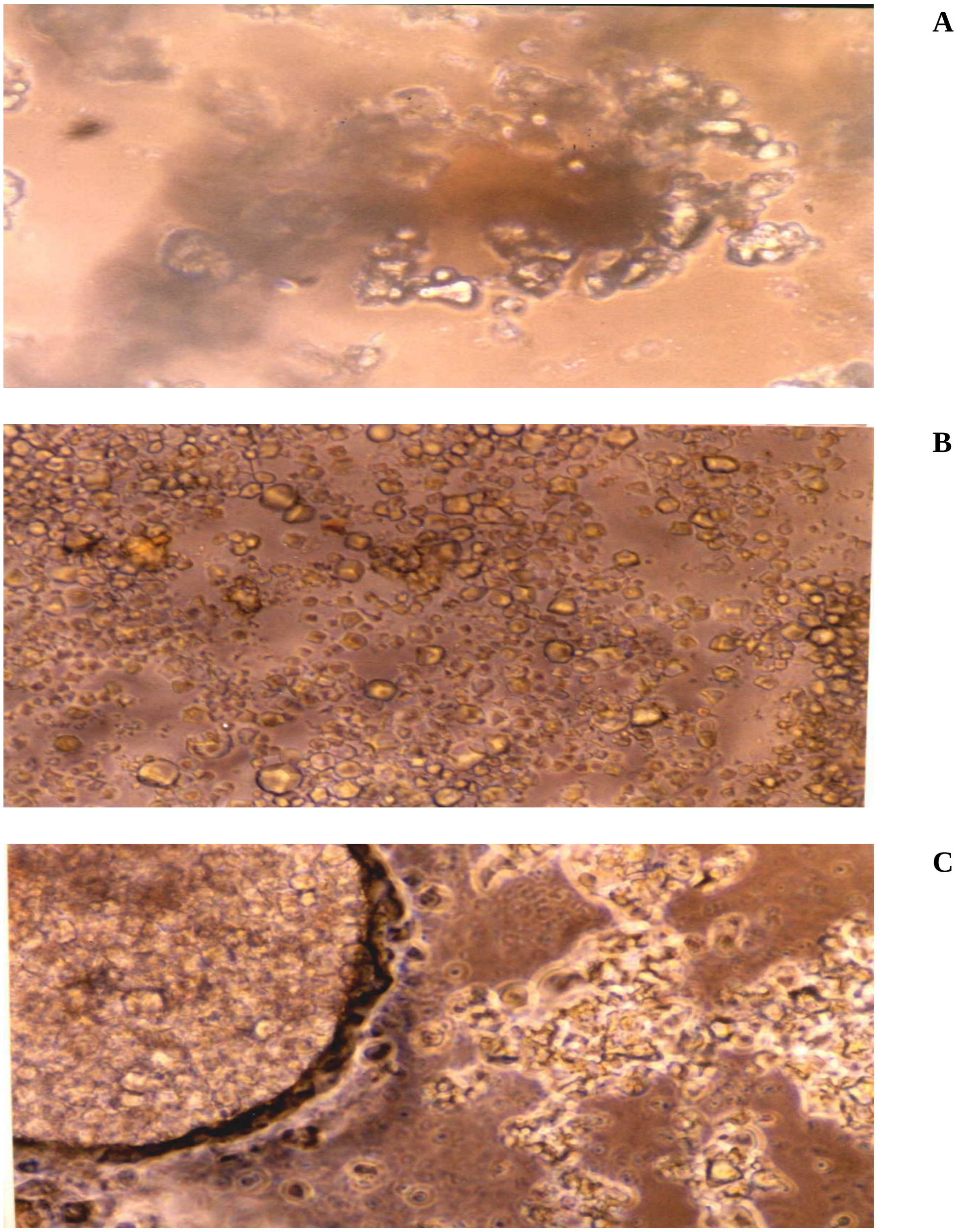

Fig. 20: Phase contrast microscopy of ragi flour,
A: Dry powder; B: Paste; C: Gelatinized
(Gelatinization was carried at 80^{o}C for 30 minutes; Magnification- 400 x)

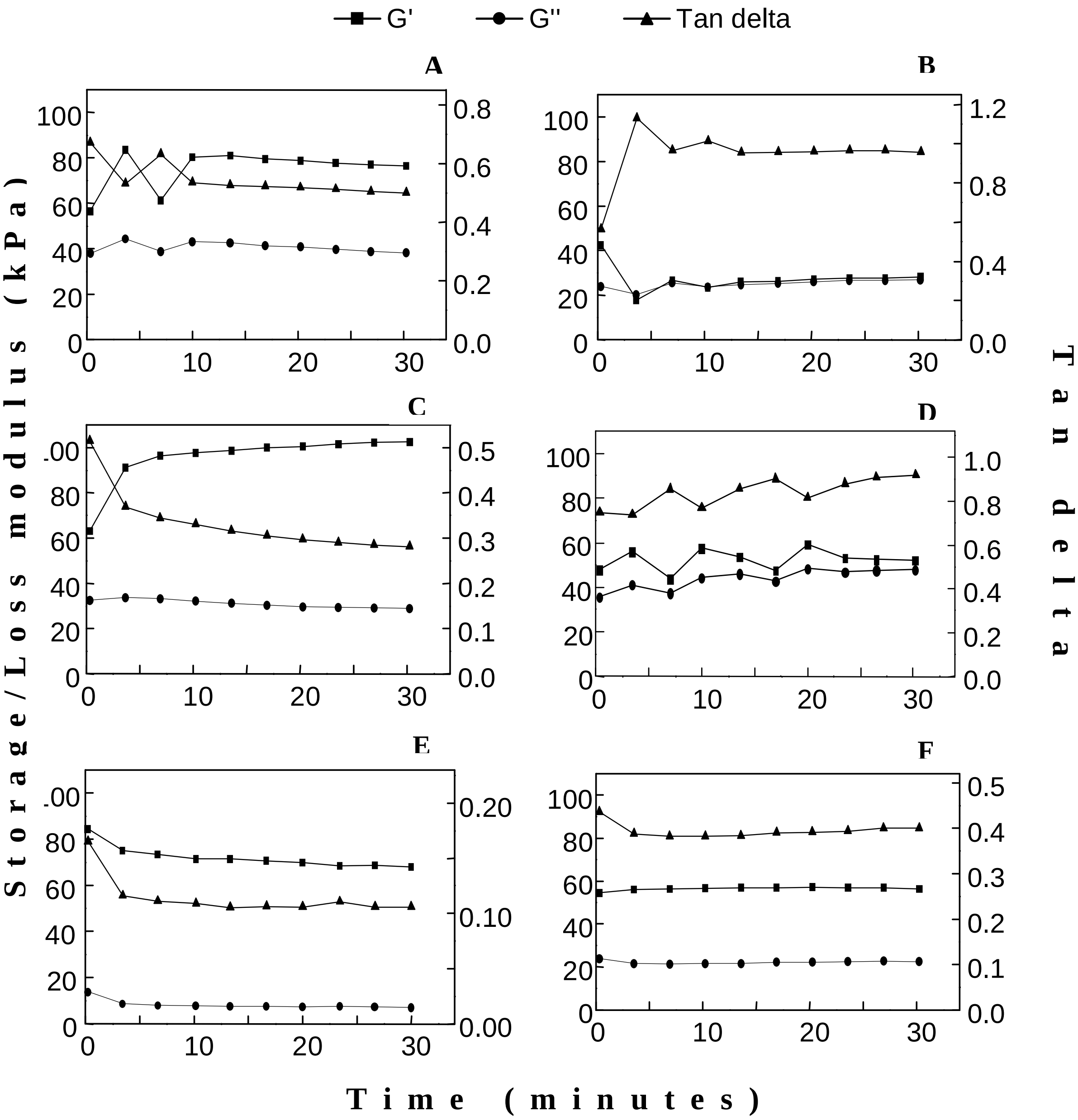

Fig. 21: **Time sweep of rice flour solution (20% w/v) A: At 60°C C: At 70°C E: At 80°C**
Rice flour with protein extract from ribbonfish B: At 60°C D: At 70°C F: At 80°C
Gap between geometry and peltier plate was set at 500 µm.

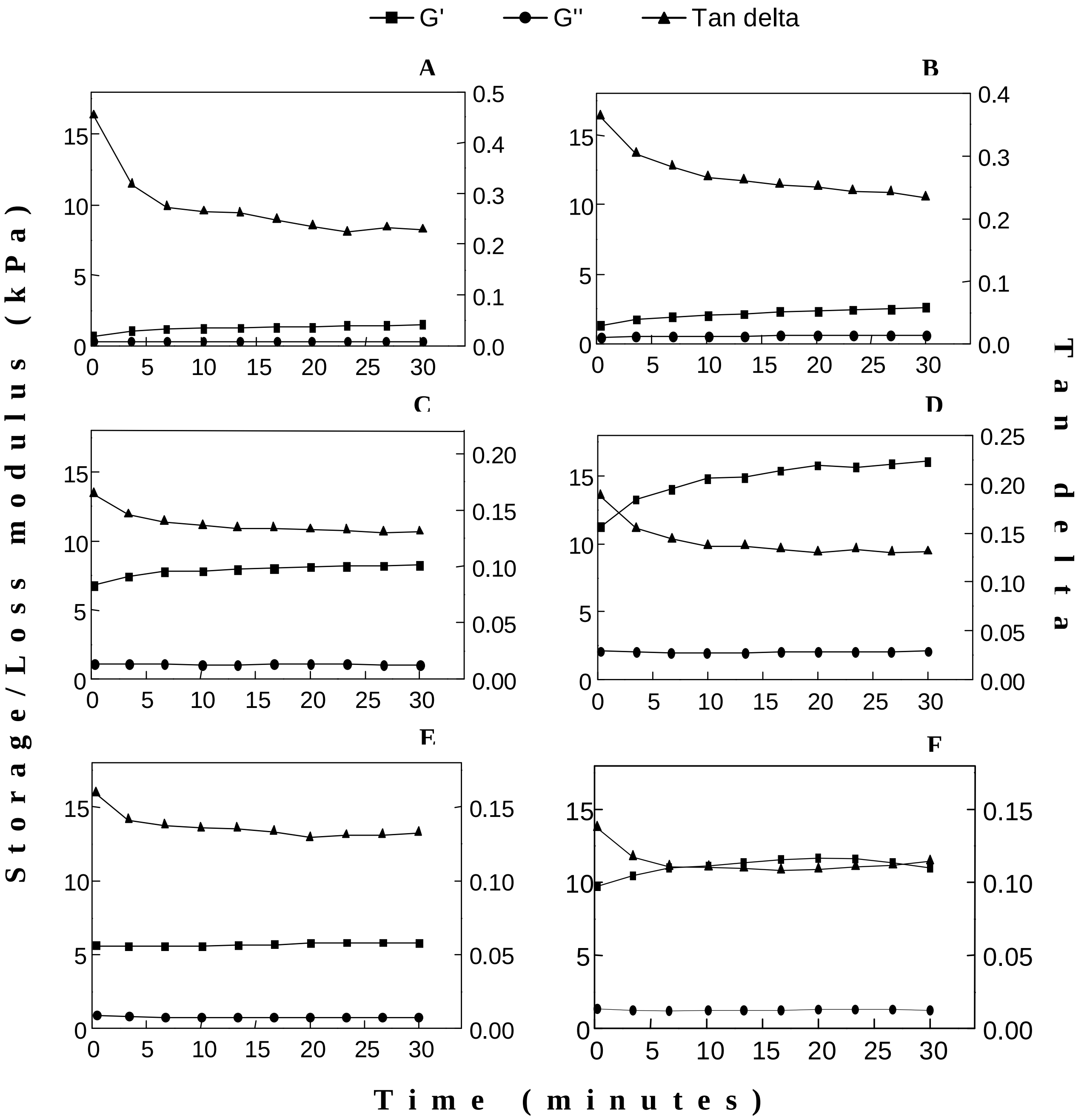

Fig. 22: Time sweep of wheat semolina solution (20% w/v) A: At 60°C; C: At 70°C; E: At 80°C Wheat semolina with protein extract from ribbonfish;B: At 60°C; D: At 70°C; F: At 80°C Gap between geometry and peltier plate was set at 500 µm.

value observed at 70°C was 16.05 KPa. With the addition of protein solution also Tan δ showed a decreasing trend.

Time sweep of ragi flour solution at 70°C and 80°C showed that G' value increased with increase in time period. At 60°C, the increase in G' was maximum during first 5 minutes of time sweep after which the values decreased (Fig. 23). The maximum value for G' (81.71 KPa) was observed at 80°C. Tan δ values showed a decreasing trend. In case of ragi flour also addition of protein solution from ribbonfish meat caused increase in G' values. The maximum G' value (134.9 KPa) was observed at 70°C at the end of 30 minutes. Tan δ values showed a decreasing trend over the time sweep period.

4.7. Extruded products

Extruded products were prepared from rice flour/ wheat semolina/ ragi flour – fish mince mixture as a function of barrel temperature, screw speed and die diameter. Barrel temperature was found to be one of the major variables affecting extruded product quality. Photographs of extruded products prepared as a function of barrel temperature from a mixture of flours (rice flour, wheat semolina and ragi flour) with fish mince is given in Fig. 24-29.

4.8. Quality evaluation of extruded products

4.8.1. Proximate composition

As a function of barrel temperature

Proximate composition of extruded products prepared, as a function of barrel temperature, from a mixture of rice flour and fish mince is given in Table 12. The moisture content in the samples with fish mince was higher than the control. There was an increase in moisture content with the increase in percentage of fish mince added irrespective of the barrel temperature. A decrease in moisture content of the extruded sample was observed with the increase in barrel temperature. The protein content varied between 8-10% in the extruded samples with fish mince, which is higher than that of the control samples. The fat content of the extruded samples with fish mince varied between 0.40 - 0.69%. There was a slight increase in fat content in the samples with increase in barrel temperature. The ash content of the extruded samples with fish content varied between 3.10% and 3.60%, which was higher than the ash content of the control samples (1.76-1.96%).

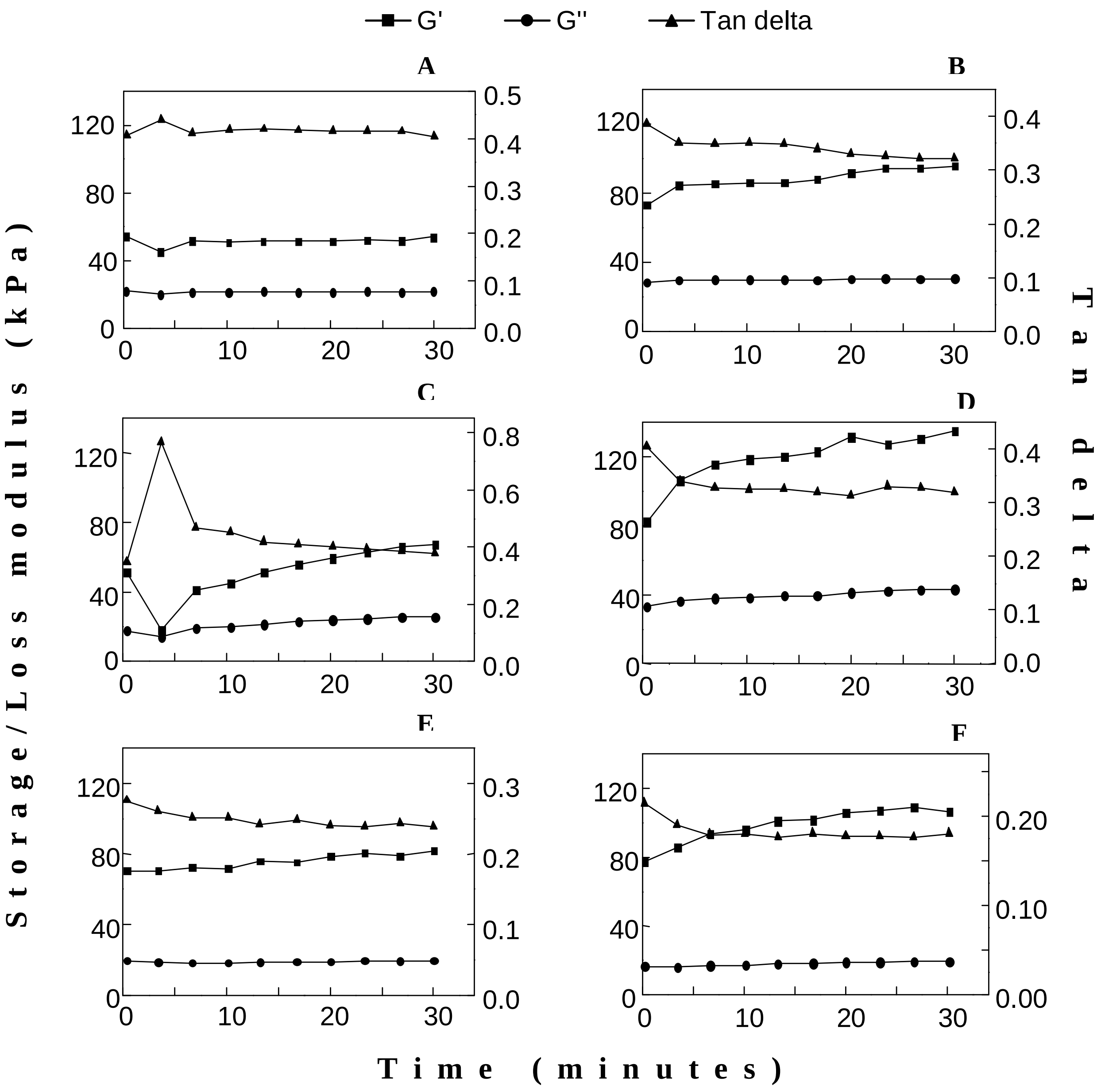

Fig. 23: Time sweep of ragi flour solution (20% w/v) A: At 60°C C: At 70°C E: At 80°C
Ragi flour with protein extract from ribbonfish B: At 60°C D: At 70°C F: At 80°C
Gap between geometry and peltier plate was set at 500 µm.

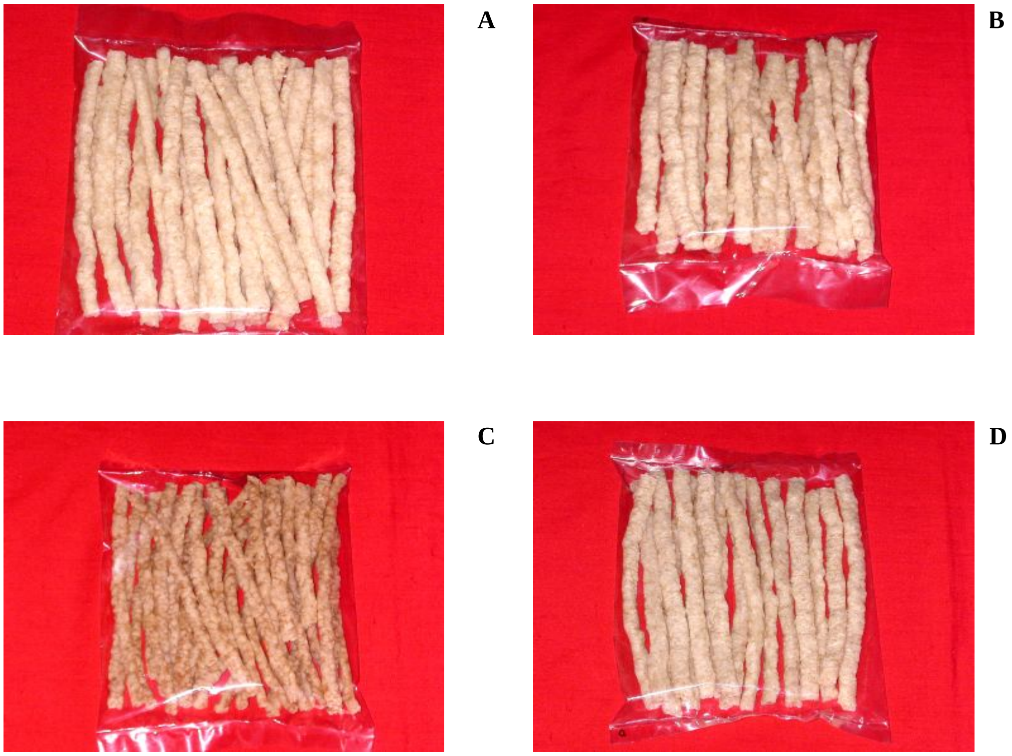

Fig. 24: Extruded products from rice flour-ribbonfish mixture
A: with 10% ribbonfish mince at 70^{o}C B: with 10% ribbonfish mince at 90^{o}C
C: with 20% ribbonfish micne at 70^{o}C D: with 20% ribbonfish mince at 90^{o}C
(Products prepared at a screw speed of 350 RPM using 4 mm die diameter)

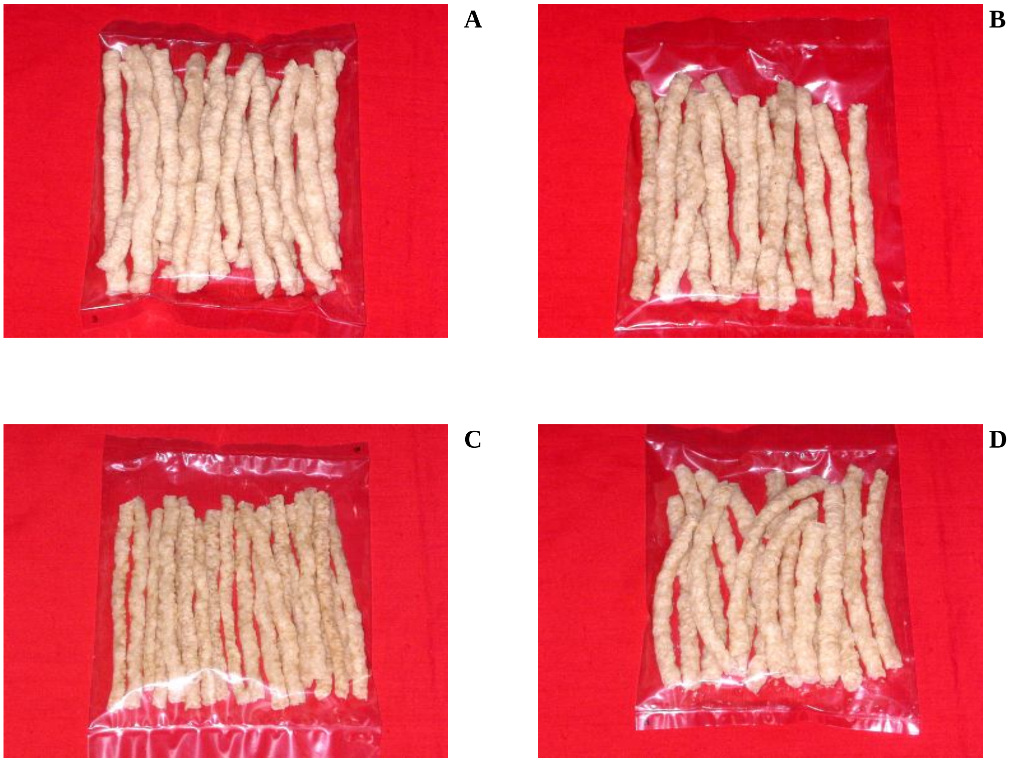

Fig. 25: Extruded products from rice flour-bull's eye fish mince mixture
A: with 10% bull's eye mince at 70^{o}C B: with 10% bull's eye mince at 90^{o}C
C: with 20% bull's eye mince at 70^{o}C D: with 20% bull's eye mince at 90^{o}C
(Products prepared at a screw speed of 350 RPM using 4 mm die diameter)

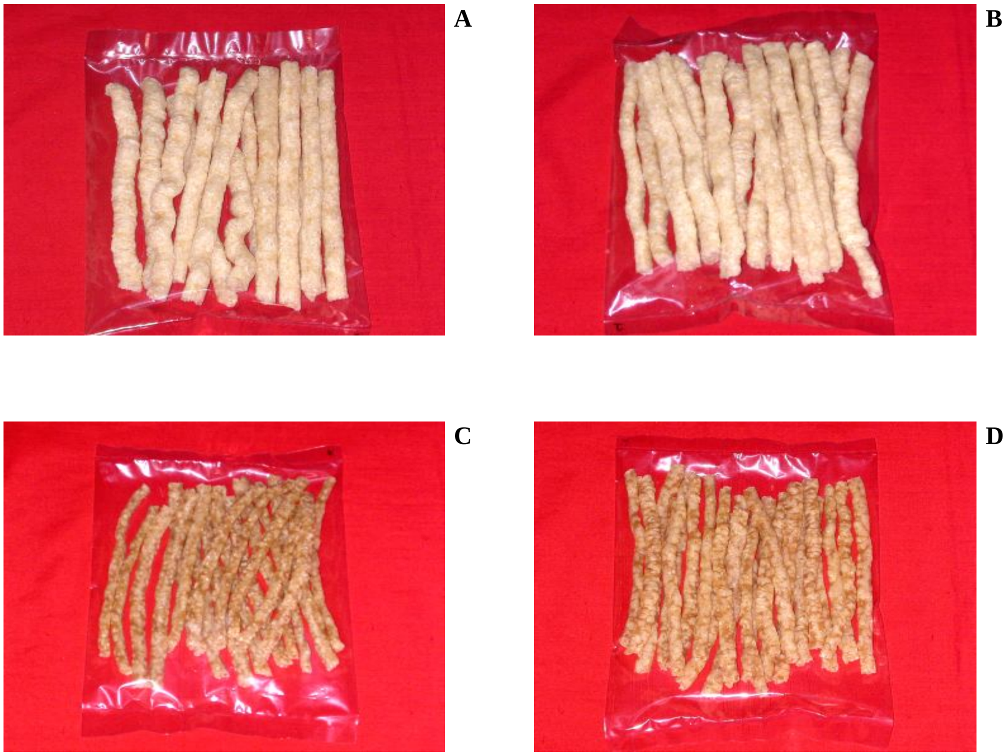

Fig. 26: Extruded products from wheat semolina-ribbonfish mince mixture
A: with 10% ribbonfish mince at 70^{o}C B: with 10% ribbonfish mince at 90^{o}C
C: with 20% ribbonfish mince at 70^{o}C D: with 20% ribbonfish mince at 90^{o}C
(Products prepared at a screw speed of 350 RPM using 4 mm die diameter)

Fig. 27: Extruded products from wheat semolina bull's eye fish mince mixture
A: with 10% bull's eye mince at 70^{o}C B: with 10% bull's eye mince at 90^{o}C
C: with 20% bull's eye mince at 70^{o}C D: with 20% bull's eye mince at 90^{o}C
(Products prepared at a screw speed of 350 RPM using 4 mm die diameter)

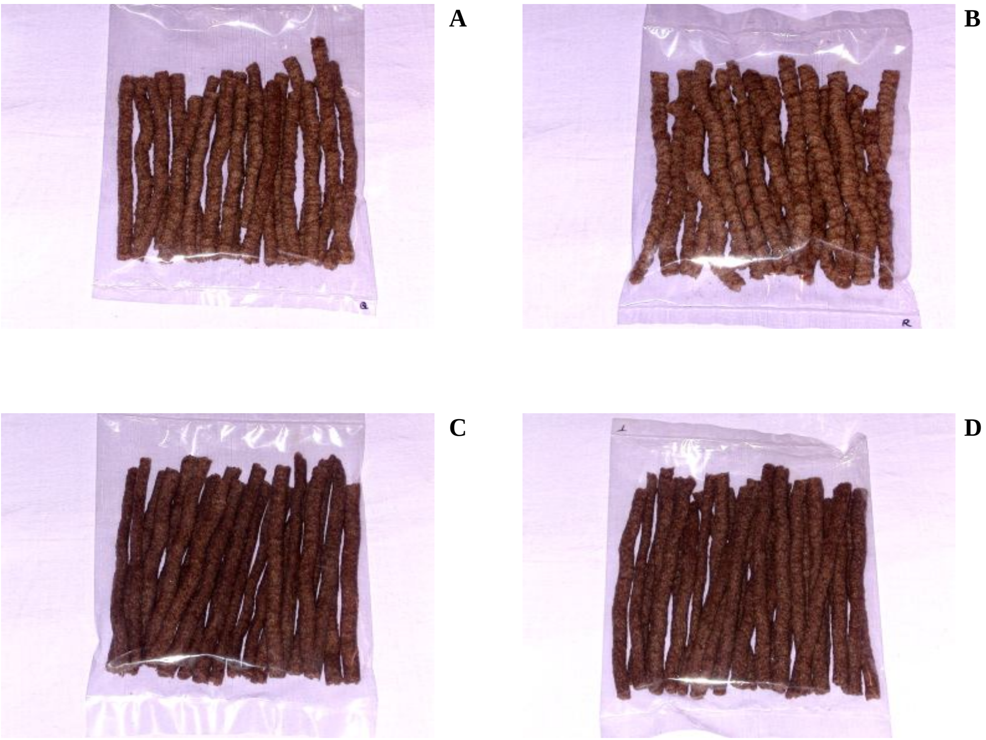

Fig. 28: Extruded products from ragi flour-ribbonfish mince mixture
A: with 10% ribbonfish mince at 70°C B: with 10% ribbonfish mince at 90°C
C: with 20% ribbonfish mince at 70°C D: with 20% ribbonfish mince at 90°C
(Products prepared at a screw speed of 350 RPM using 4 mm die diameter)

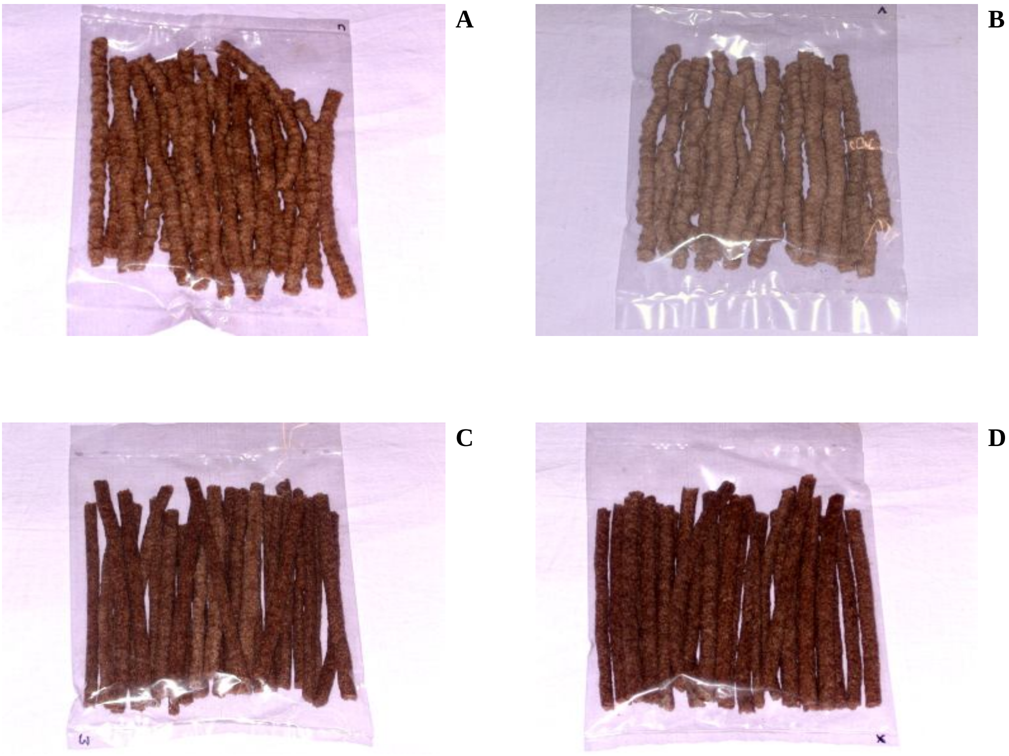

Fig. 29: Extruded products from ragi flour-bull’s eye fish mince mixture
A: with 10% bull’s eye mince at 70°C B: with 10% bull’s eye mince at 90°C
C: with 20% bull’s eye mince at 70°C D: with 20% bull’s eye mince at 90°C
(Products prepared at a screw speed of 350 RPM using 4 mm die diameter)

Table 12. Proximate composition of extruded products[†] as a function of barrel temperature and percentage of fish meat

Flour used	Para-meters	Barrel temperature												
		70^{o}C					90^{o}C					120^{o}C		
		C*	10% RF*	20% RF	10% BE*	20% BE	C*	10% RF	20% RF	10% BE	20% BE	C*	10% RF	10% BE
Rice flour	Moisture	7.11	9.02	12.81	7.92	12.10	5.73	8.96	9.36	7.51	10.88	4.10	8.00	7.11
	Protein	6.73	8.01	9.57	7.84	9.66	6.55	8.07	10.50	8.18	10.31	6.51	8.47	8.26
	Fat	0.27	0.41	0.40	0.46	0.67	0.31	0.54	0.43	0.51	0.69	0.33	0.56	0.57
	Ash	1.93	3.37	3.42	3.60	3.62	1.96	3.18	3.14	3.45	3.45	1.76	2.87	3.10
Wheat flour	Moisture	4.05	7.89	11.28	8.22	11.71	3.57	7.87	10.57	7.40	10.31	3.26	7.55	7.01
	Protein	10.55	12.25	12.45	12.69	12.89	10.51	13.36	13.00	12.85	12.91	10.10	13.40	12.93
	Fat	0.40	0.58	0.89	0.50	0.56	0.45	0.60	0.91	0.53	0.59	0.51	0.61	0.57
	Ash	2.89	2.92	3.22	2.45	2.42	2.85	3.45	3.11	2.94	2.75	2.73	3.13	3.10
Ragi flour	Moisture	3.40	8.32	12.72	9.48	12.87	3.39	7.72	11.17	8.44	12.45	3.34	7.51	7.55
	Protein	7.06	8.66	10.27	8.52	9.16	7.12	8.89	10.77	8.57	9.33	7.23	8.88	8.61
	Fat	0.24	0.38	0.51	0.48	0.65	0.36	0.43	0.57	0.69	0.72	0.42	0.61	0.49
	Ash	4.05	3.69	4.83	4.75	4.41	3.92	4.35	4.73	4.56	3.43	4.02	4.57	4.23

* Control samples (without fish mince); RF: Ribbonfish mince; BE: Bull’s eye fish mince

[†] Products were prepared at constant screw speed of 350 RPM using a die diameter of 4 mm

Proximate composition of extruded products from wheat semolina-fish mince mixture prepared as a function of barrel temperature is given in Table 12. The moisture content in the extruded products showed a decreasing trend with increase in barrel temperature. The moisture content in the control samples was between 3.26% and 4.05%. Protein content was slightly higher than that of extruded products from rice and ragi flour. Protein content in the control samples was between 10-10.5%. Fat content showed a slight increase with increase in barrel temperature. Ash content in the wheat control samples was higher than that of control of rice flour.

Moisture, protein, fat and ash content of extruded products prepared as a function of barrel temperature from ragi flour-fish mince mixture is given in Table 12. There was an increase in moisture content with an increase in fish percentage, irrespective of the barrel temperature. The moisture content in all the samples decreased with increase in the barrel temperature. Protein content was slightly higher than that of rice products but was less than that of wheat semolina based products. The fat content showed an increasing trend with an increase in barrel temperature. Ash content in all the samples was higher than those from rice flour or wheat semolina based products.

As a function of screw speed

Proximate composition of extruded products from a mixture of rice flour and 10% ribbonfish mince prepared as a function of screw speed is given in Table 13. The moisture content showed an increasing trend both in the control (without fish mince) and sample with an increase in screw speed. The protein content of extruded samples with fish minces and rice flour varied between 7.65-7.86% as a function of screw speed. There was a marginal decrease in fat content with increase in screw speed.

Proximate composition of wheat semolina based extruded products prepared as a function of screw speed is given in Table 13. There was a marginal increase in moisture content with increase in screw speed. Protein content was higher in wheat semolina based products compared to rice/ragi flour based products. Fat content decreased marginally with the increase in screw speed.

Proximate composition of ragi based products prepared as a function of screw speed is given in Table 13. The trend in the changes of moisture and fat content was

Table 13. Proximate composition of extruded products* as a function of screw speed

Flour used	Parameters	Screw speed					
		300 RPM		400 RPM		500 RPM	
		Control	10% RF	Control	10% RF	Control	10% RF
Rice flour	Moisture	3.76	7.62	4.24	7.79	5.90	7.81
	Protein	6.64	7.65	6.25	7.71	6.13	7.86
	Fat	0.31	0.55	0.24	0.52	0.19	0.48
	Ash	2.10	2.61	2.22	2.65	1.95	2.79
Wheat flour	Moisture	6.03	8.56	6.59	8.57	7.94	8.95
	Protein	10.09	11.76	10.77	11.79	10.62	12.08
	Fat	0.40	0.43	0.37	0.41	0.31	0.35
	Ash	3.39	3.37	3.50	3.30	3.28	3.34
Ragi flour	Moisture	2.99	7.46	3.03	7.68	4.53	7.89
	Protein	6.78	8.65	6.65	7.89	5.92	7.68
	Fat	0.36	0.39	0.33	0.36	0.24	0.30
	Ash	3.91	4.26	4.32	4.07	4.42	3.72

RF: Ribbonfish mince

* Only ribbonfish mince at 10% level was used for this study. The products were prepared at a constant barrel temperature of 90°C using a die diameter of 4 mm.

similar to that rice flour and wheat semolina based products. Ash content was slightly higher than rice flour or wheat semolina based products.

As a function of die diameter

Proximate composition of extruded products from a mixture of rice flour/wheat semolina/ragi flour and 10% ribbonfish mince prepared as a function of die diameter is given in Table 14. In extruded rice flour based products the moisture content showed an increasing trend both in the control and sample (with fish mince) with an increase in die diameter. The protein content in the samples was around 8% and was higher than the control. There was a marginal decrease in fat content with increase in die diameter.

In the case of wheat semolina based extruded products, the trend in the changes of moisture and fat were similar to rice based products. Protein content was higher than rice or ragi based products.

In ragi based extruded products the changes in moisture and fat content were similar to that of rice flour and wheat semolina based products. Ash content was higher than rice flour or wheat semolina based products.

4.8.2. Aminoacid composition of extruded rice products with added fish mince

Aminoacid analysis of extruded products from a mixture of rice flour –fish mince mixture showed a higher proportion of glutamic acid, alanine, tryptophan, leucine and lysine (Table 15). Unlike fresh fish amino acid pattern, the extruded product showed a higher proportion of tryptophan. The proportion of lysine was higher showing the nutritional importance of extruded products. There was no much difference in the amino acid proportion with the percentage of fish mince added except some amino acids like lysine, which was higher with higher percentage of fish mince. Majority of amino acids, especially lysine and glutamic acid content was higher in products prepared with ribbonfish mince than with bull’s eye fish mince. The presence of all the essential amino acid in the sample emphases the nutritional importance of extruded products.

Table 14. Proximate composition of extruded products* as a function of die diameter

Flour used	Parameters	Die diameter					
		3 mm		4 mm		5 mm	
		Control	10% RF	Control	10% RF	Control	10% RF
Rice flour	Moisture	3.65	8.12	5.73	8.96	9.40	11.40
	Protein	6.77	8.35	6.55	8.07	6.41	7.85
	Fat	0.38	0.57	0.31	0.54	0.30	0.46
	Ash	1.97	3.05	1.96	3.18	2.52	3.25
Wheat flour	Moisture	3.30	7.71	3.57	7.87	5.29	9.09
	Protein	9.94	12.31	10.51	13.36	10.88	12.28
	Fat	0.46	0.54	0.45	0.60	0.30	0.37
	Ash	2.96	2.73	2.85	3.45	2.68	2.80
Ragi flour	Moisture	3.01	7.32	3.39	7.72	3.76	7.93
	Protein	7.17	8.91	7.12	8.89	6.70	7.68
	Fat	0.39	0.48	0.36	0.43	0.24	0.38
	Ash	4.04	4.33	3.92	4.35	3.91	4.42

RF: Ribbonfish mince

* Only ribbonfish mince at 10% level was used for this study. The products were prepared at a constant screw speed of 350 RPM at a barrel temperature of 90°C.

Table 15. Aminoacid composition of extruded products* from mixture of rice flour and fish mince

Aminoacid	With 10% ribbonfish mince	With 20% ribbonfish mince	With 10% bull's eye fish mince	With 20% bull's eye fish mince
Asp	6.46 ± 0.32	6.52 ± 0.10	5.94 ± 0.22	5.73 ± 0.19
Glu	12.75 ± 0.41	12.14 ± 0.17	11.80 ± 0.28	11.74 ± 0.22
Hpro	0.85 ± 0.82	0.55 ± 0.10	0.29 ± 0.01	0.85 ± 0.20
Ser	4.69 ± 0.23	4.65 ± 0.11	4.74 ± 0.24	4.53 ± 0.17
Gly	6.93 ± 0.22	6.79 ± 0.28	6.58 ± 0.30	6.31 ± 0.16
His	1.90 ± 0.25	2.09 ± 0.06	2.13 ± 0.02	2.06 ± 0.01
Arg	4.58 ± 0.08	4.26 ± 0.07	4.41 ± 0.08	4.42 ± 0.05
Thr	3.43 ± 0.03	3.62 ± 0.03	3.38 ± 0.03	3.22 ± 0.01
Ala	11.91 ± 0.20	11.40 ± 0.14	11.03 ± 0.15	10.85 ± 0.09
Pro	3.16 ± 0.70	3.14 ± 0.47	3.91 ± 0.61	3.74 ± 0.33
Tyr	2.58 ± 0.42	2.34 ± 0.72	2.33 ± 0.36	2.14 ± 0.35
Val	6.41 ± 0.35	6.44 ± 0.15	6.55 ± 0.25	5.99 ± 0.35
Met	2.63 ± 0.21	2.68 ± 0.17	2.78 ± 0.27	5.54 ± 0.29
Cys	1.12 ± 0.16	1.25 ± 0.03	1.47 ± 0.05	1.27 ± 0.04
Ileu	4.05 ± 0.27	4.22 ± 0.13	3.84 ± 0.37	3.99 ± 0.15
Leu	7.92 ± 0.46	7.69 ± 0.02	7.66 ± 0.42	7.21 ± 0.07
Phe	4.17 ± 0.31	4.10 ± 0.29	4.28 ± 0.42	3.86 ± 0.17
Try	8.59 ± 0.45	8.86 ± 0.48	11.18 ± 0.58	10.66 ± 0.51
Lys	5.86 ± 0.18	7.27 ± 0.01	5.71 ± 0.03	5.88 ± 0.05

(as % aminoacid) (Given values represent Mean ± standard deviation)

*Products prepared at a screw speed of 350 RPM and barrel temperature of 90^{o}C using a die diameter of 4 mm.

4.8.3. Expansion ratio of extruded products

As a function of barrel temperature

Expansion ratio of rice flour based extruded products as a function of barrel temperature is given in Table 16 A, Fig. 30. The expansion ratio of all the samples increased with increase in barrel temperature irrespective of the percentage of fish mince added. Extruded products from rice flour with 10% ribbonfish mince showed an expansion ratio above the control at any given barrel temperature and the maximum expansion (375%) was obtained at 120^{o}C. Extruded products with 20% fish mince prepared at 70^{o}C gave the least expansion. Expansion ratio of extruded products prepared with the addition of bull’s eye fish mince was lower compared to that with ribbonfish mince irrespective of the barrel temperature.

Wheat semolina-fish mince mixture was extruded at different barrel temperature and the expansion ratio is given in Table 16 A, Fig. 30. Extruded products from wheat semolina with 10% bull’s eye fish mince showed an expansion ratio above the control at any given temperature and the maximum (350%) was at 120^{o}C. With 10% ribbonfish mince the products showed an expansion ratio (307%) equal to that of control only at 120^{o}C. Products prepared with 20% ribbonfish at 70^{o}C showed the least expansion (200%).

Expansion ratio of extruded ragi flour-fish mince mixture at different barrel temperature is given in Table 16 A, Fig. 30. At any given barrel temperature expansion ratio was significantly ($P<0.05$) higher for extruded products from ragi flour with 10% bull’s eye fish mince than the control and the maximum expansion ratio (268%) was obtained at 120^{o}C (Table 16 B). The expansion ratio of ragi based extruded products were lower when compared with that of rice flour or wheat semolina based products. There was no much difference in the expansion ratio of products prepared with 20% ribbonfish mince or bull’s eye fish mince.

As a function of screw speed

Expansion ratio of extruded products from rice flour, wheat semolina and ragi flour with 10% ribbonfish mince prepared at 90^{o}C using a 4 mm die diameter as a function of screw speed is given in Table 17 A, Fig. 31. The addition of ribbonfish mince reduced the

Table 16 A. Expansion ratio of extruded products* from flour-fish mince mixture as a function of barrel temperature

Flour	Percentage of fish	Barrel Temperature		
		70°C	90°C	120°C
Rice control	Nil	227	295	299
Rice	10% Ribbonfish	289	311	375
	20% Ribbonfish	202	294	ND
	10% Bull’s eye fish	265	268	326
	20% Bull’s eye fish	191	222	ND
Wheat control	Nil	275	295	307
Wheat	10% Ribbonfish	215	283	307
	20% Ribbonfish	200	225	ND
	10% Bull’s eye fish	313	318	350
	20% Bull’s eye fish	250	275	ND
Ragi control	Nil	223	231	245
Ragi	10% Ribbonfish	175	213	223
	20% Ribbonfish	163	175	ND
	10% Bull’s eye fish	238	263	268
	20% Bull’s eye fish	175	175	ND

ND: Not Determined

* Products prepared at a constant screw speed of 350 RPM using a 4 mm die diameter

Table 16 B. F- ratios from results of Analysis of Variance of expansion ratio as a function of barrel temperature

Sources of variation	Rice flour		Wheat semolina		Ragi flour	
	RF	BE	RF	BE	RF	BE
Between Temperature	6.37	3.66	4.04	11.26	4.77	9.57
Between samples	8.02	0.39	1.71	33.28*	9.73	22.58*

Statistical analysis has been carried out between control samples and products with 10% fish meat only. (RF: Ribbonfish meat; BE: Bull’s eye fish meat)

* Denotes significant difference ($P<0.05$)

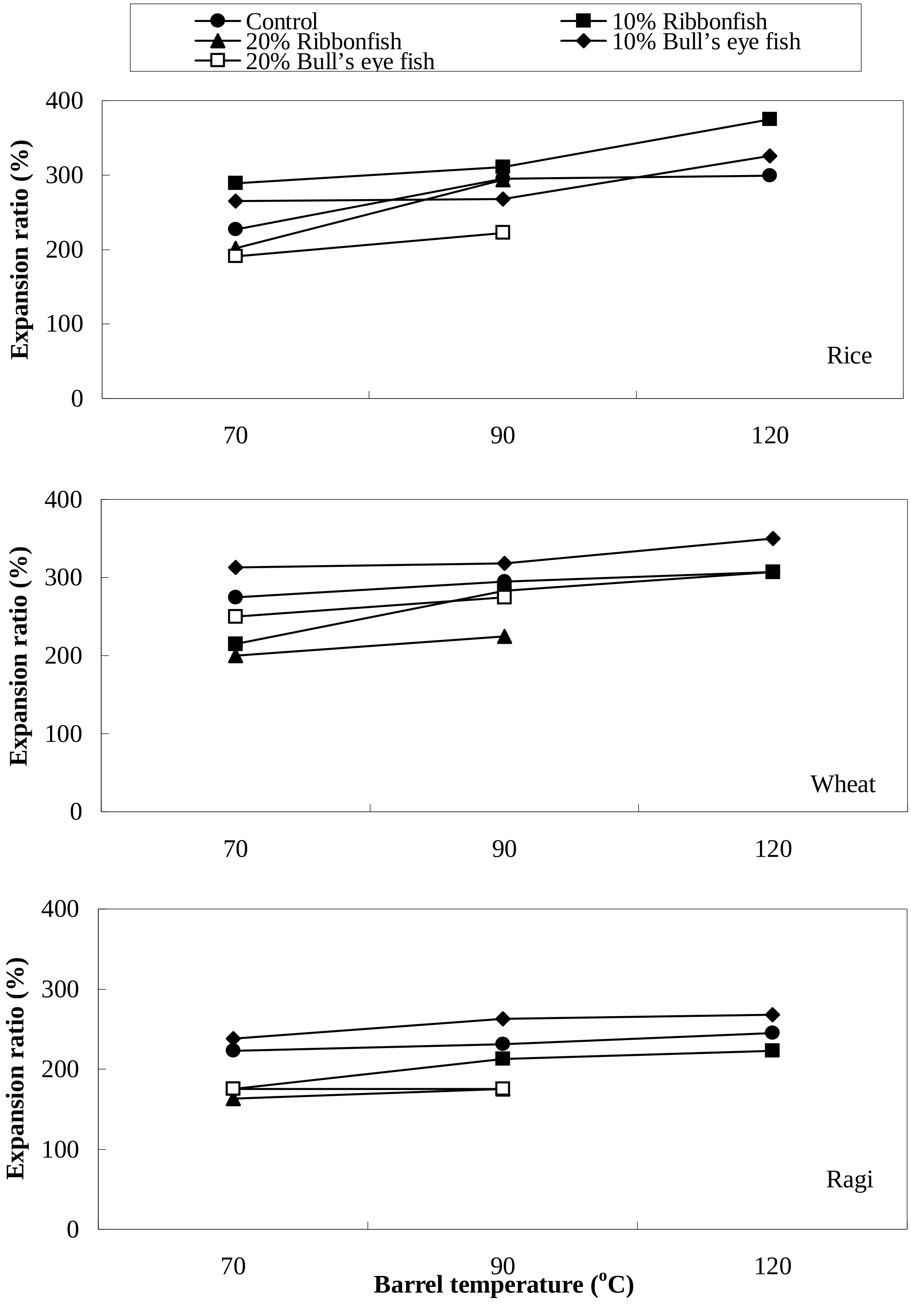

Fig. 30: Expansion ratio of extruded products from different flours prepared at a constant screw speed of 350 RPM using a die diameter of 4 mm as a function of barrel temperature

Table 17 A. Expansion ratio of extruded products* from flour-fish mince mixture as a function of screw speed

Raw material	Screw speed		
	300 RPM	400 RPM	500 RPM
Rice Control	307	303	283
Rice+10% Ribbonfish	303	278	268
Wheat Control	263	267	271
Wheat+10% Ribbonfish	303	295	279
Ragi Control	231	235	239
Ragi+10% Ribbonfish	213	200	188

* **Products were prepared at a constant barrel temperature of 90°C using a 4 mm die diameter**

Table 17 B. F- ratios from results of Analysis of Variance of expansion ratio as a function of screw speed

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Screw speed	7.88	0.26	0.26
Between samples	5.84	7.36	13.23

RF: Products with 10% Ribbonfish meat

No significant difference between samples as a function of screw speed ($P<0.05$)

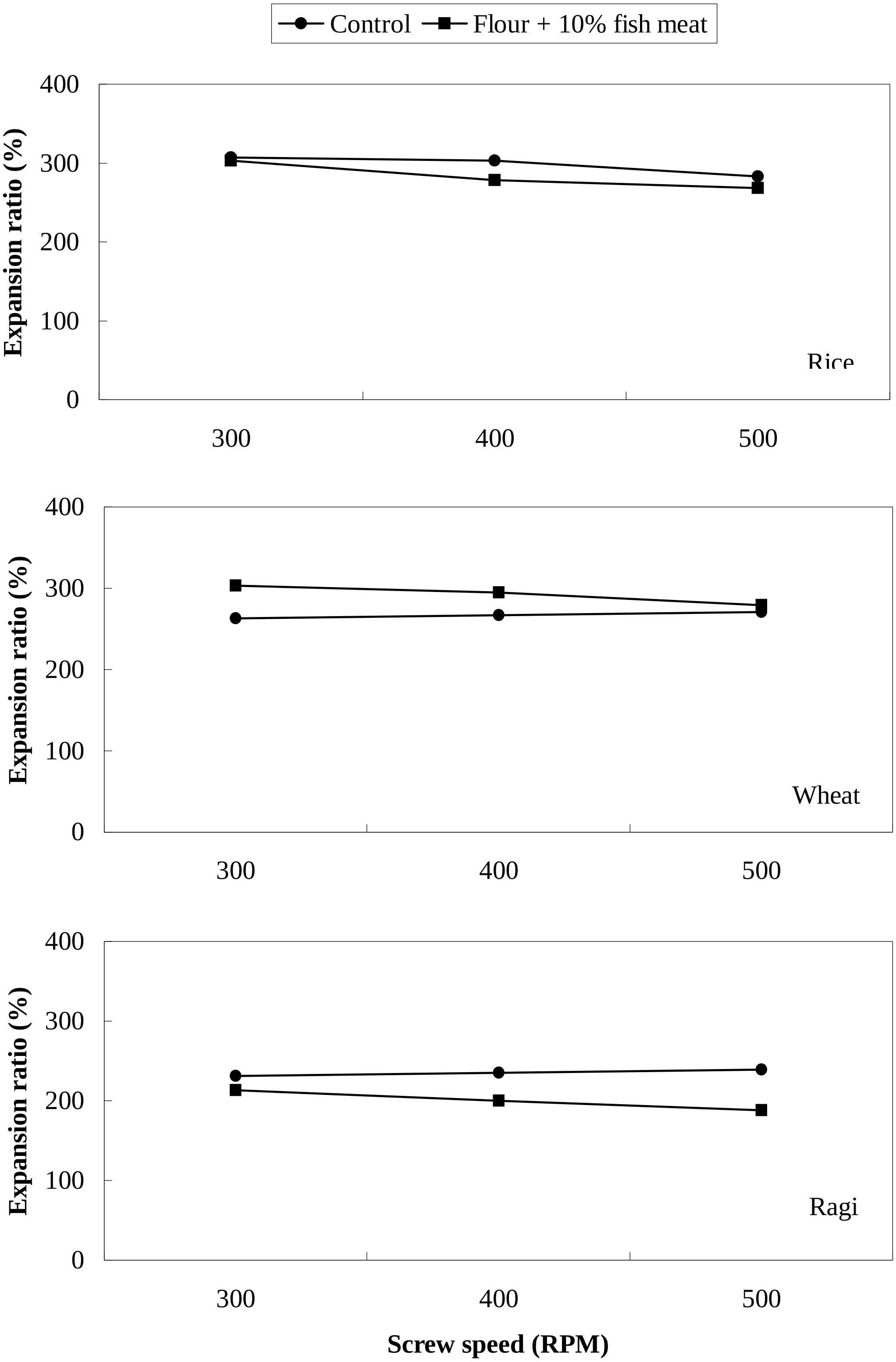

Fig. 31: Expansion ratio of extruded products from flour-fish meat mixture as a function of screw speed (Products were prepared at a screw speed of 350 RPM using 4 mm die diameter)

expansion ratio of rice/ragi flour at any given screw speed. In the case of extruded products from wheat semolina, the expansion ratio was more with addition of fish mince than the control. The expansion ratio decreased with increase in screw speed in all the samples containing fish mince. However, ragi flour control samples showed a marginal increase in expansion ratio with increase in screw speed. Expansion ratio of extruded products from rice flour/wheat semolina and ragi flour did not show any significant ($P<0.05$) difference as a function of screw speed or with the addition of 10% fish mince (Table 17 B).

As a function of die diameter

Expansion ratio of extruded products from rice flour, wheat semolina and ragi flour with 10% ribbonfish mince prepared, at a constant screw speed of 350 RPM and barrel temperature of 90^{o}C, as a function of die diameter is given in Table 18 A, Fig. 32. From the data it is clear that increase in die diameter resulted in decrease in product expansion irrespective of the flours used. There was no much difference between the expansion ratio of the products with and without fish at any given die diameters. The maximum expansion ratio (425%) with the addition of fish mince was shown by rice flour extruded using a die diameter of 3 mm and the minimum was shown by ragi flour with fish mince extruded using 5 mm die diameter (210%). Rice and ragi flour based extruded products showed a significant ($P<0.05$) reduction with increase in die diameter (Table 18 B).

4.8.4. Water absorption capacity of extruded products

As a function of barrel temperature

The water absorption capacity of extruded products from rice flour with varying percentage of fish mince, barrel temperature and fish species is given in Table 19 A, Fig. 33. In all the samples water absorption capacity increased with increase in barrel temperature. Water absorption was higher for the sample prepared with addition of 10% bull’s eye fish mince. Almost all samples had water absorption above the control. With increase in percentage of fish mince, the water absorption capacity of extruded products decreased. Water absorption capacity of wheat semolina based extruded products is given in Table 19 A, Fig. 33. In case of extruded products from wheat semolina, control sample was having more water absorption capacity. Among the samples with fish mince content, the water absorption capacity was maximum for sample with 10% bull’s eye fish mince and the least was for sample with 20% ribbonfish mince. Here also, WAC increased with

Table 18 A. Expansion ratio of extruded products* from flour-fish mince mixture as a function of die diameter

Raw material	Die diameter		
	3 mm	4 mm	5 mm
Rice Control	441	295	255
Rice+10% Ribbonfish	425	311	223
Wheat Control	398	295	223
Wheat+10% Ribbonfish	340	283	229
Ragi Control	255	231	217
Ragi+10% Ribbonfish	250	213	210

* Products were prepared at a constant screw speed of 350 RPM and barrel temperature of 90°C

Table 18 B. F- ratios from results of Analysis of Variance of expansion ratio as a function of die diameter

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Die diameter	65.43*	18.86	34.33*
Between samples	0.57	1.25	6.12

RF: Ribbonfish meat

* Denotes significant difference ($P<0.05$)

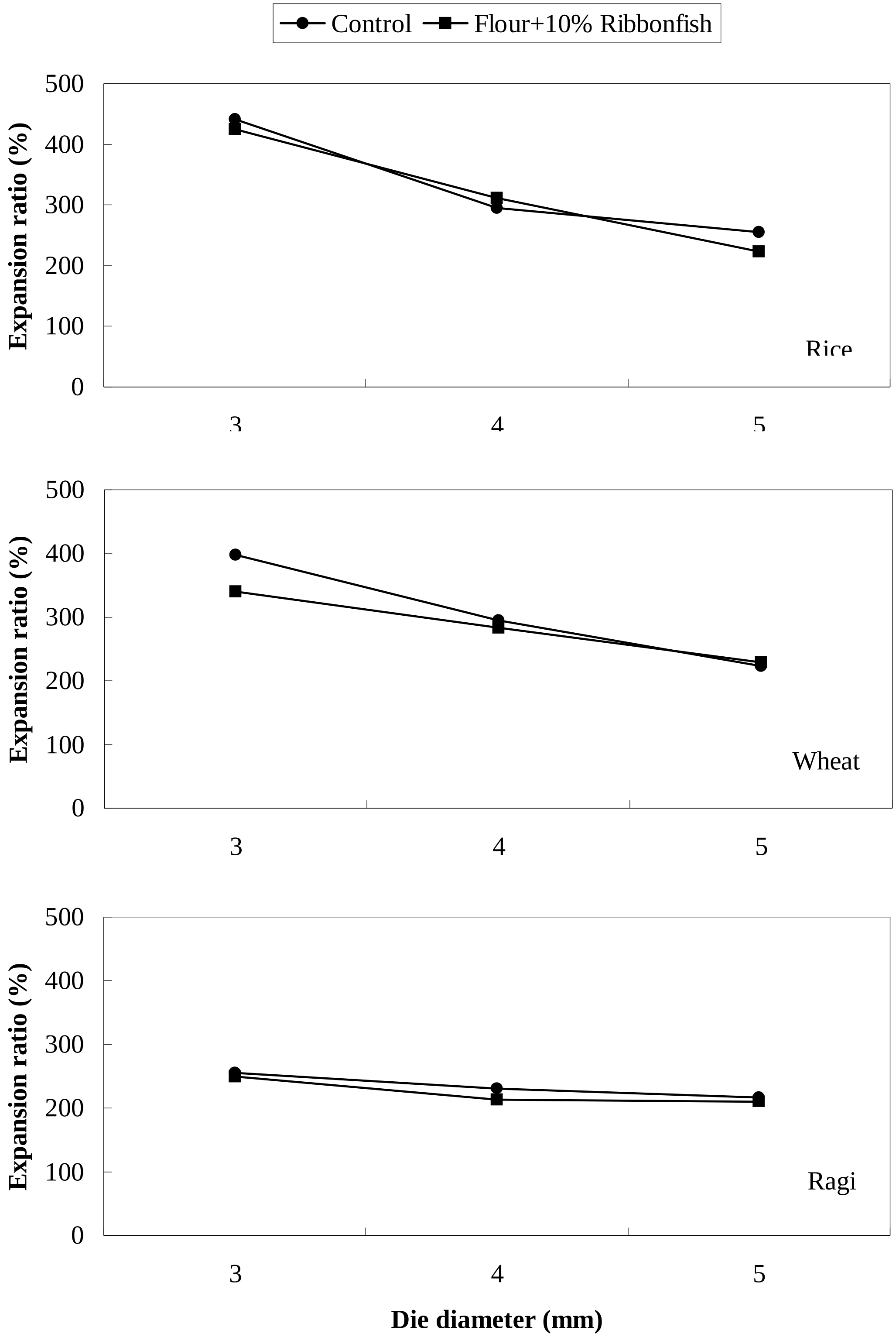

Fig. 32: **Expansion ratio of flour-fish mince mixture extrudates as a function of die diameter (Products were prepared at a constant barrel temperature of 90°C and screw speed of 350 RPM)**

Table 19 A. Water absorption capacity of extruded products* from mixture of flour-fish mince as a function of barrel temperature

Flour	Percentage of fish	Barrel temperature		
		70°C	90°C	120°C
Rice	Control	4.78	4.84	4.89
	10% Ribbonfish	5.06	6.54	6.99
	20% Ribbonfish	4.56	5.64	ND
	10% Bull's eye fish	5.40	6.81	7.06
	20% Bull's eye fish	4.34	5.72	ND
Wheat	Control	6.85	6.97	7.04
	10% Ribbonfish	3.69	5.64	5.74
	20% Ribbonfish	2.26	2.62	ND
	10% Bull's eye fish	5.74	5.91	6.07
	20% Bull's eye fish	3.49	4.64	ND
Ragi	Control	3.90	4.38	4.85
	10% Ribbonfish	3.18	3.58	4.01
	20% Ribbonfish	2.58	3.17	ND
	10% Bull's eye fish	4.16	4.70	4.97
	20% Bull's eye fish	2.85	2.98	ND

ND: Not Determined

* Products prepared at a constant screw speed of 350 RPM using 4 mm die diameter

Table 19 B. F- ratios from results of Analysis of Variance of water absorption capacity as a function of barrel temperature

Sources of variation	Rice flour		Wheat semolina		Ragi flour	
	RF	BE	RF	BE	RF	BE
Between Temperature	1.24	1.26	1.37	13.49	212*	74.00*
Between samples	6.06	10.62	9.84	652*	497*	15.51

Statistical analysis has been carried out between control samples and products with 10% fish meat only. (RF: Ribbonfish meat; BE: Bull's eye fish meat)

* Denotes significant difference ($P<0.05$)

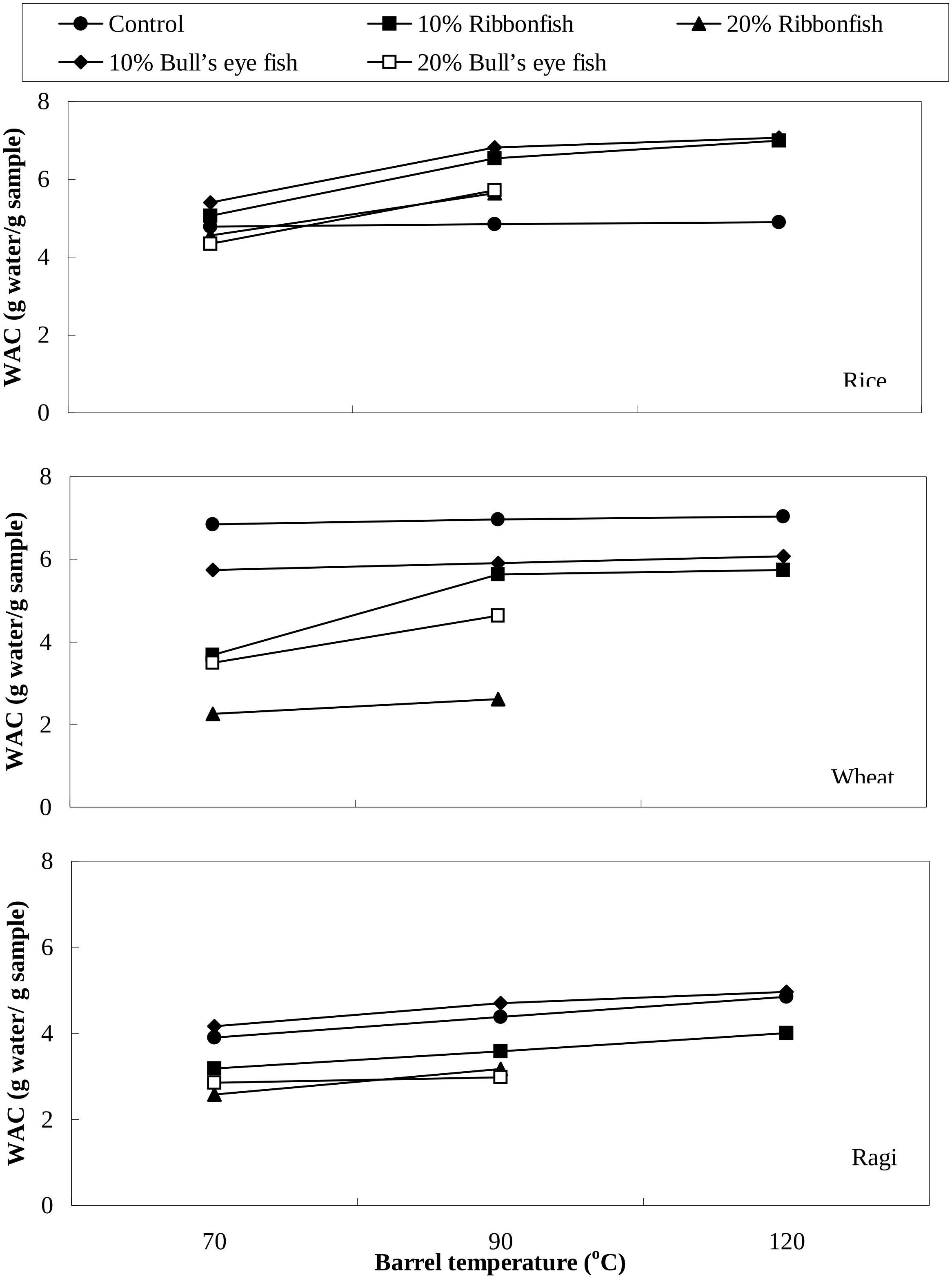

Fig. 33: Water absorption capacity of extruded products from flour-fish mince mixture as a function of barrel temperature (Products were prepared at a constant screw speed of 350 RPM using a die diameter of 4 mm

increase in barrel temperature. The water absorption capacity of ragi based extruded products is given in Table 19 A, Fig. 33. Extruded products with 10% bull's eye fish mince showed significantly ($P<0.05$) higher water absorption capacity than the control irrespective of the barrel temperature (Table 19 B). However with 20% fish mince, the water absorption capacity of products reduced.

As a function of screw speed

Water absorption capacity of extruded products from rice flour, wheat semolina and ragi flour with 10% ribbonfish mince prepared at 90^{o}C using a 4 mm die diameter as a function of screw speed is given in Table 20 A, Fig. 34. Water absorption capacity decreased with the addition of ribbonfish mince in rice based extruded products. In general WAC increased with increase in screw speed. The water absorption capacity of wheat semolina based extruded products showed that the values for products prepared with fish mince were significantly ($P<0.05$) higher than the control samples (Table 20 B). The water absorption capacity of ragi based extruded products didn't show considerable difference between samples with and without fish mince.

As a function of die diameter

Water absorption capacity of extruded products from rice flour, wheat semolina and ragi flour with 10% ribbonfish mince prepared, at a constant screw speed of 350 RPM and barrel temperature of 90^{o}C, as a function of die diameter is given in Table 21 A, Fig. 35. Addition of fish mince caused an increase in WAC in the case of rice flour at any given die diameters. However, the increase was not significant ($P<0.05$). With an increase in die diameter, WAC of rice based products increased. But in the case of products from wheat semolina, WAC of products with fish mince was less than the control except using 5 mm die diameter. Increase in die diameter caused a decrease in WAC in the case of wheat semolina based extruded products. There was no significant ($P<0.05$) difference in WAC of control and sample in the case of products prepared from ragi flour but WAC increased for the products when the die diameter was increased (Table 21 B).

Table 20 A. Water absorption capacity of extruded products* as a function of screw speed

Raw material	Screw speed		
	300 RPM	400 RPM	500 RPM
Rice Control	5.03	5.86	5.98
Rice + 10% Ribbonfish	4.36	4.99	5.80
Wheat Control	3.53	4.65	4.81
Wheat + 10% Ribbonfish	6.02	6.49	7.26
Ragi Control	3.62	3.95	5.30
Ragi + 10% Ribbonfish	3.56	3.89	4.37

* Products prepared at a constant barrel temperature of 90°C using a die diameter of 4 mm

Table 20 B. F- ratios from results of Analysis of Variance of water absorption capacity as a function of screw speed

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Screw speed	11.51	12.23	6.59
Between samples	7.82	115.47*	1.45

RF: Products with ribbonfish meat

* Denotes significant difference ($P<0.05$)

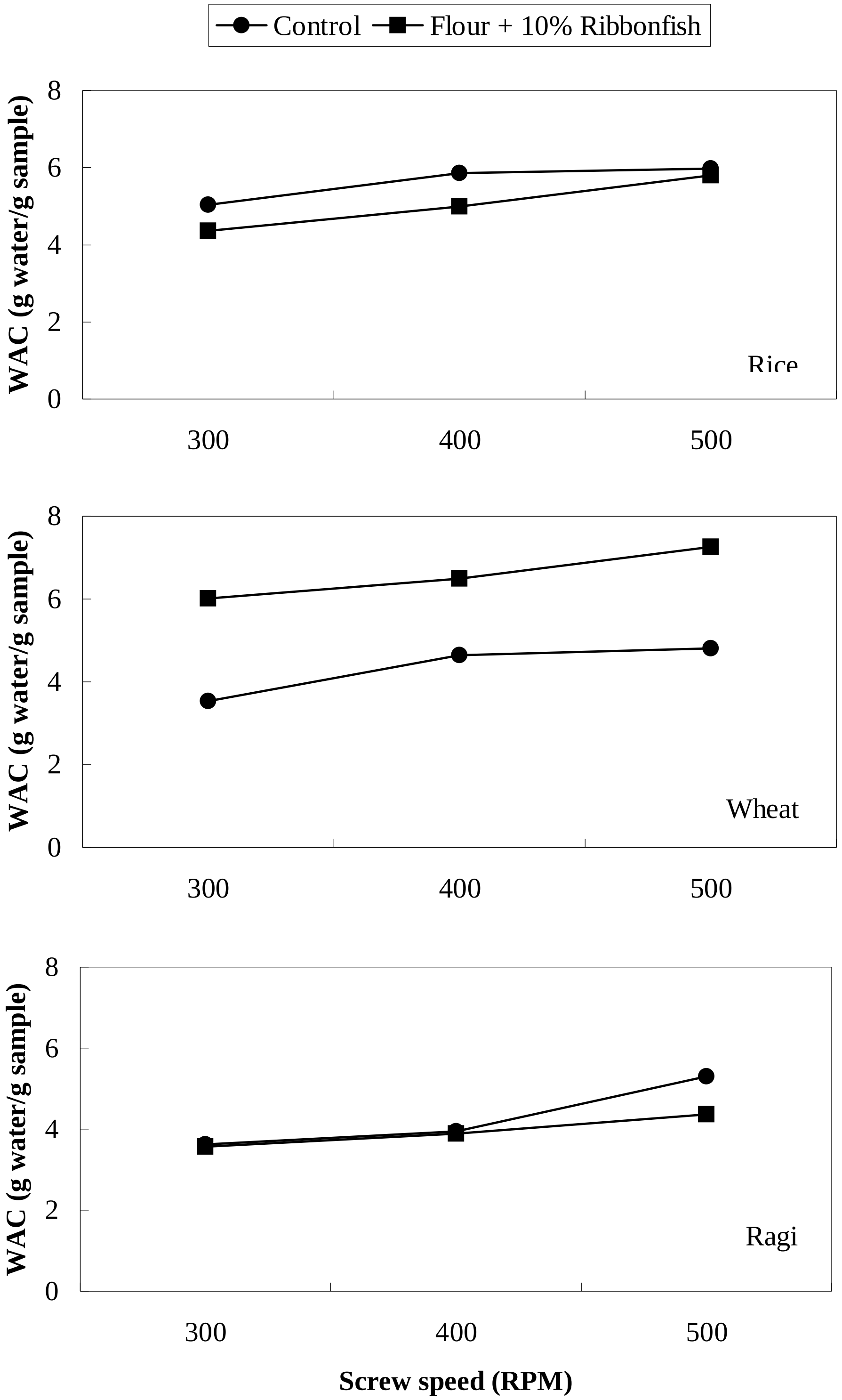

Fig. 34: **Water absorption capacity of extruded products from flour-fish mince mixture as a function of screw speed (Products prepared at a constant barrel temperature of 90°C using a 4 mm die diameter)**

Table 21 A. Water absorption capacity of extruded products as a function of die diameter

A

Raw material	Die diameter		
	3 mm	4 mm	5 mm
Rice Control	4.38	4.84	5.03
Rice + 10% Ribbonfish	5.01	5.40	6.54
Wheat Control	7.21	6.97	3.04
Wheat + 10% Ribbonfish	6.01	5.74	4.54
Ragi Control	3.71	4.38	5.32
Ragi + 10% Ribbonfish	4.01	4.34	4.36

***** Products prepared at a constant barrel temperature of 90°C and screw speed of 350 RPM

Table 21 B. F - ratios from results of Analysis of Variance of water absorption capacity as a function of die diameter

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Die diameter	4.30	3.96	2.26
Between samples	8.67	0.12	0.38

RF: Products with 10% Ribbonfish meat
No significant difference between samples as a function of die diameter ($P<0.05$)

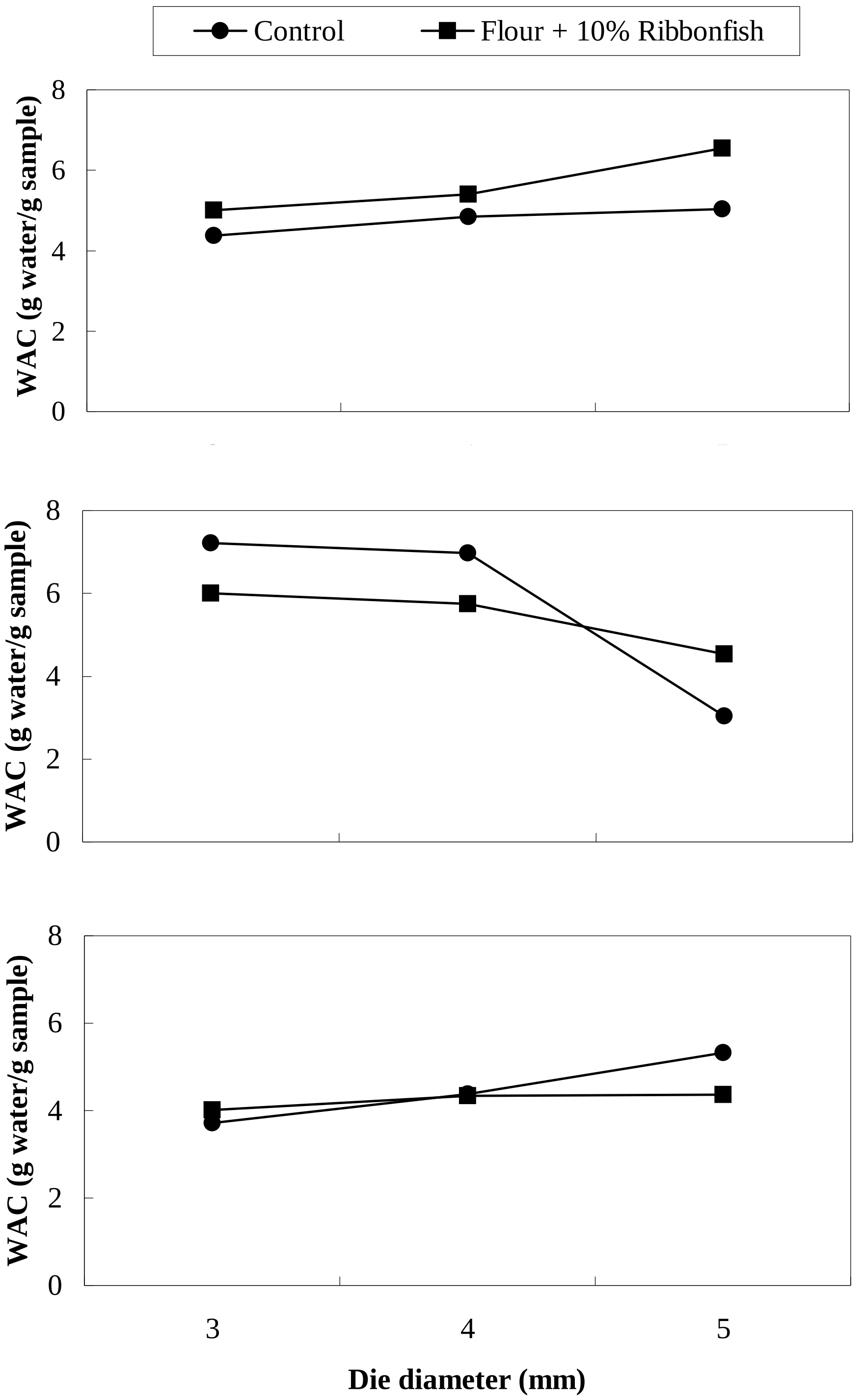

Fig. 35: Water absorption capacity of extruded products from flour-ribbonfish mince mixture as a function of die diameter (Products prepared at a constant barrel temperature of 90^{o}C and screw speed of 350 RPM)

4.8.5. Color analysis of extruded products

4.8.5.1. As a function of barrel temperature

Rice flour-fish mince mixture

The color values (L, a* and b*) for extruded products were measured as a function of barrel temperature. The L values of extruded products from rice flour-fish mince mixture (Table 22A, Fig. 36A) revealed that the control sample was having the highest value at 70°C and 90°C, while it was the lowest at 120°C. Among the samples with fish mince, the L value was higher for samples with 10% ribbonfish mince. The lowest L value (50.96) was for the product with 20% bull's eye fish mince. The L values didn't show much difference at a barrel temperature of 90°C.

The a* values of extruded products prepared from rice flour-fish mince mixture, as a function of barrel temperature is given in Table 22B, Fig. 36B. The data revealed that the sample with the least L value showed the highest a* value. At 20% level of bull's eye mince the L value was least. Products with 10% fish mince showed an increasing trend in a* values with an increase in barrel temperature. However, the increment was not significant at 5% level of significance (Table 25 A).

The b* values of the extruded products from mixture of rice flour-fish mince, prepared as a function of barrel temperature is given in Table 22C, Fig. 36C. At 70°C the highest b* value was for the sample with 20% bull's eye fish mince. At 120°C the value for the sample with 10% ribbonfish mince showed the maximum value. From 70°C to 90°C, almost all samples showed a decreasing trend in b* values.

Wheat semolina-fish mince mixture

The L values of extruded products from wheat semolina-fish mince mixture (Table 23A, Fig. 37A) revealed that there was no much difference in the values of control or with 10% or 20% fish mince. The highest L value (77.07) was shown by products with 20% ribbonfish extruded at 90°C. In general, for all the samples there was an increase in L values with an increase in barrel temperature.

The a* values of extruded products prepared, from wheat semolina-fish mince mixture as a function of barrel temperature is given in Table 23B, Fig, 37B. In all the

Table 22. Color values of extruded products from rice flour-fish mince mixture as a function of barrel temperature; A: L* values; B: a* values; C: b* values

A

Flour	Percentage of fish	70°C	90°C	120°C
Rice	Control	68.17 (0.57)	73.26 (0.32)	55.22 (1.63)
	10% Ribbonfish	63.32 (0.60)	67.08 (1.36)	76.58 (1.52)
	20% Ribbonfish	63.79 (0.45)	63.82 (0.99)	ND
	10% Bull's eye fish	63.33 (0.49)	66.59 (1.02)	68.64 (0.19)
	20% Bull's eye fish	50.96 (0.25)	70.76 (0.07)	ND

B

Flour	Percentage of fish	70°C	90°C	120°C
Rice	Control	1.93 (0.02)	0.87 (0.02)	2.19 (0.04)
	10% Ribbonfish	1.07 (0.03)	1.69 (0.01)	1.95 (0.05)
	20% Ribbonfish	1.93 (0.04)	0.85 (0.01)	ND
	10% Bull's eye fish	1.44 (0.01)	1.41 (0.04)	1.78 (0.03)
	20% Bull's eye fish	2.37 (0.04)	0.99 (0.01)	ND

C

Flour	Percentage of fish	70°C	90°C	120°C
Rice	Control	12.26 (0.19)	11.19 (0.12)	10.83 (0.12)
	10% Ribbonfish	13.38 (0.14)	12.13 (0.09)	16.91 (0.16)
	20% Ribbonfish	12.75 (0.06)	11.91 (0.21)	ND
	10% Bull's eye fish	13.05 (0.13)	13.80 (0.32)	12.67 (0.08)
	20% Bull's eye fish	14.43 (0.03)	13.41 (0.06)	ND

ND: Not Determined; Values in parenthesis represent standard deviation
(Products prepared at a constant screw speed of 350 RPM using a die diameter of 4 mm)

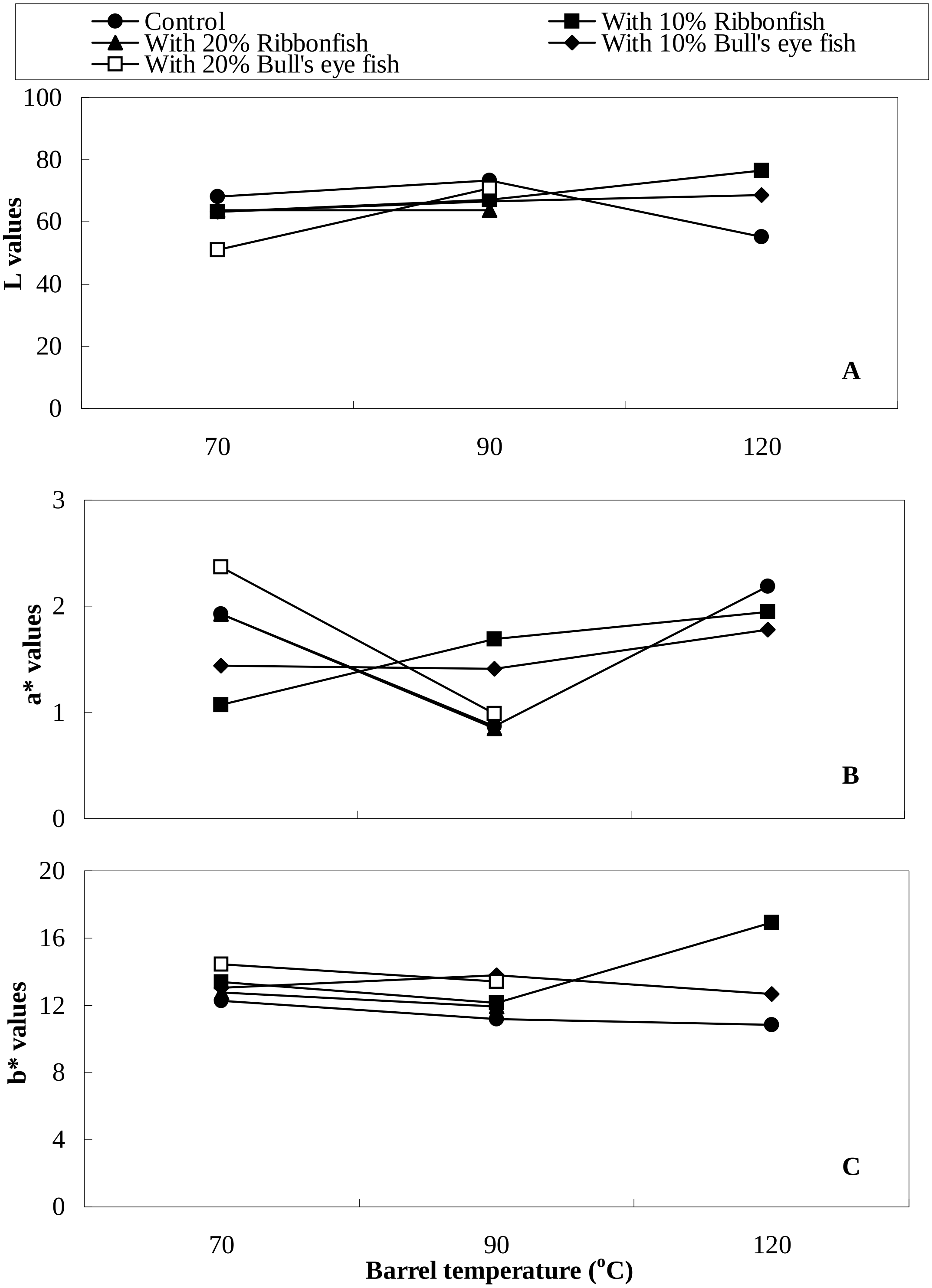

Fig. 36: Color values of extruded rice flour-fish mince mixture as a function of barrel temperature A: L values; B: a* values C: b* values (Products prepared at a constant screw speed of 350 RPM using a 4 mm die diameter)

Table 23. Color values of extruded products from wheat semolina-fish mince mixture as a function of barrel temperature; A: L* values; B: a* values; C: b* values

A

Flour	Percentage of fish	70°C	90°C	120°C
Wheat	Control	67.43 (0.24)	68.02 (0.90)	69.19 (2.05)
	10% Ribbonfish	66.45 (0.53)	66.62 (0.98)	78.22 (0.19)
	20% Ribbonfish	67.28 (0.67)	77.07 (0.46)	ND
	10% Bull's eye fish	70.53 (1.55)	70.97 (0.39)	74.10 (0.15)
	20% Bull's eye fish	65.31 (0.06)	66.01 (0.23)	ND

B

Flour	Percentage of fish	70°C	90°C	120°C
Wheat	Control	0.90 (0.01)	1.24 (0.09)	1.34 (0.04)
	10% Ribbonfish	1.70 (0.02)	1.71 (0.02)	1.37 (0.03)
	20% Ribbonfish	0.90 (0.03)	0.86 (0.03)	ND
	10% Bull's eye fish	1.58 (0.03)	1.70 (0.03)	1.95 (0.05)
	20% Bull's eye fish	0.94 (0.05)	1.00 (0.03)	ND

C

Flour	Percentage of fish	70°C	90°C	120°C
Wheat	Control	15.12 (0.03)	15.35 (0.29)	18.50 (0.18)
	10% Ribbonfish	16.71 (0.15)	16.58 (0.19)	16.86 (0.04)
	20% Ribbonfish	10.11 (0.23)	11.44 (0.09)	ND
	10% Bull's eye fish	15.70 (0.21)	16.93 (0.09)	15.51 (0.03)
	20% Bull's eye fish	14.47 (0.11)	14.52 (0.09)	ND

ND: Not determined; Values in parenthesis represent standard deviation

(Products prepared at a constant screw speed of 350 RPM using a die diameter of 4 mm)

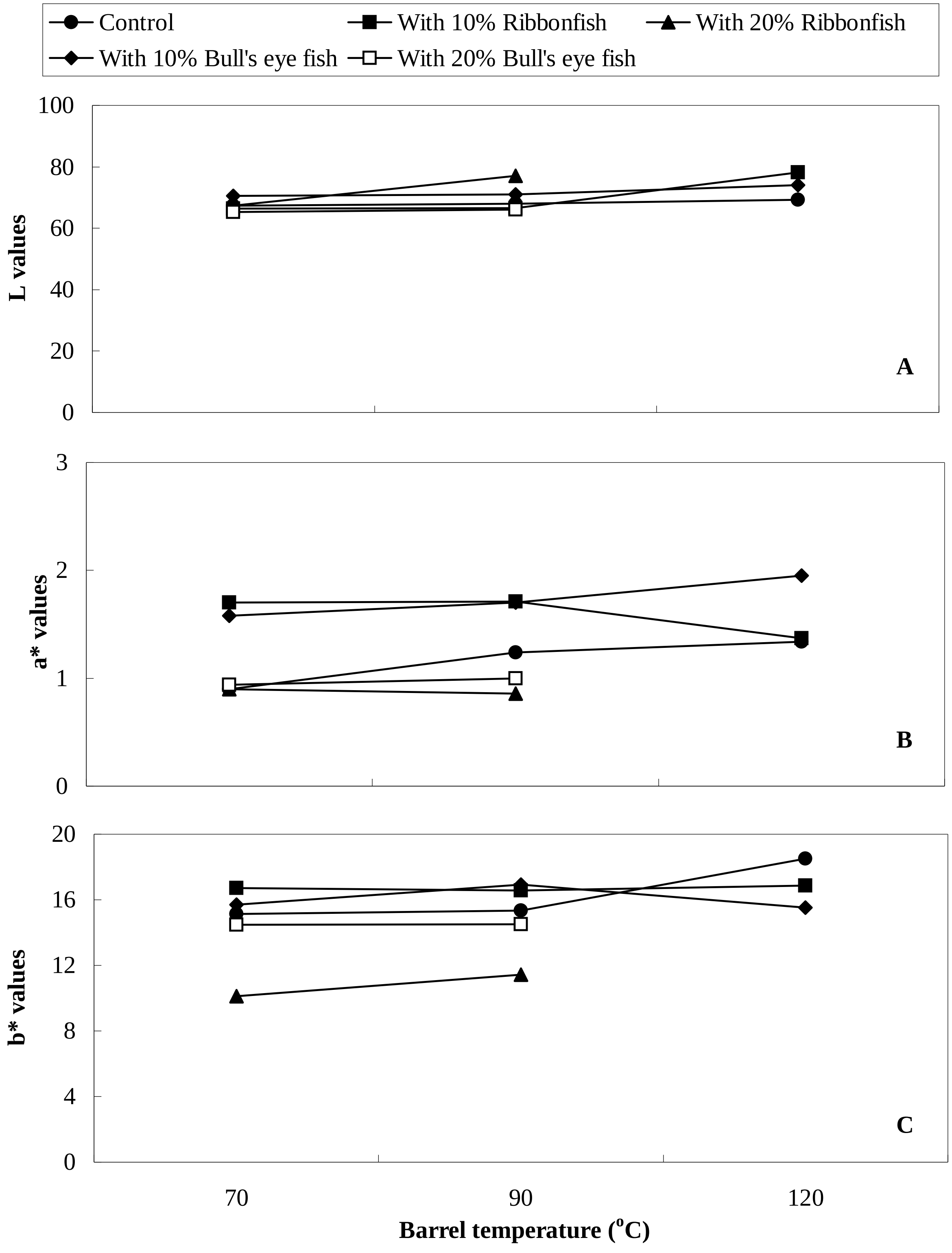

Fig. 37: Color values extruded wheat semolina-fish mince mixture as a function of temperature A: L* values; B: a* values; C: b* values (Products were prepared at a constant screw speed of 350 RPM using a 4 mm die diameter

samples there was inverse relation between L and a* values. With increase in fish mince content there was a reduction in a* values. The highest value (1.70) at 70°C was shown by the product with 10% ribbonfish mince but when the barrel temperature increased to 120°C the product with 10% bull's eye fish mince showed the highest value (1.95). Addition of 10% bull's eye fish mince caused significant ($P<0.05$) difference in L and a* values of wheat semolina based extruded products (Table 25 B).

The b* values of the extruded products from mixture of wheat semolina-fish mince prepared as a function of barrel temperature is given in Table 23C, Fig. 37C. The least value (14.52) was recorded for the products prepared with 20% ribbonfish mince at 90°C. The product with 10% ribbonfish mince showed the highest value at 70°C.

Ragi flour-fish mince mixture

The L values of extruded products from ragi flour-fish mince mixture prepared as a function of barrel temperature is given in Table 24A, Fig. 38A. The L values of extruded products from ragi are comparatively lower than that of rice flour or wheat semolina based products. There was no significant difference in the L values between different samples especially at 70°C and 120°C. The highest value (53.15) was shown by products with 20% ribbonfish mince prepared at 90°C. The products prepared with 10% ribbonfish mince irrespective of the barrel temperature showed the lowest value.

The a* values of extruded products prepared from ragi flour-fish mince mixture, as a function of barrel temperature is given in Table 24B, Fig. 38B. The products with 10% ribbonfish mince, which was having the lowest L value, showed the highest a* value. With increase in percentage of fish mince there was a decrease in a* values.

The b* values of the extruded products from mixture of ragi flour-fish mince prepared as a function of barrel temperature is given in Table 24C, Fig. 38C. The difference in b* values were not significant with respect to barrel temperature. With the addition of fish mince b* values also decreased. Value for the control sample was the least (11.12). Higher b* values (13.85) were shown by the products containing 10% ribbonfish mince which had lowest L value. Addition of 10% ribbon/bull's eye fish mince caused significant ($P<0.05$) increase in a* and b* values in ragi flour based extruded products (Table 25 C).

Table 24. Color values of extruded products from ragi flour-fish mince mixture as a function of barrel temperature; A: L* values; B: a* values; C: b* values

A

Flour	Percentage of fish	70°C	90°C	120°C
Ragi	Control	42.19 (0.21)	46.10 (0.32)	43.57 (0.10)
	10% Ribbonfish	39.98 (0.80)	40.73 (0.03)	42.25 (0.09)
	20% Ribbonfish	44.12 (1.08)	49.78 (0.26)	ND
	10% Bull’s eye fish	42.94 (0.18)	43.01 (0.43)	43.67 (0.10)
	20% Bull’s eye fish	43.02 (0.42)	53.15 (0.15)	ND

B

Flour	Percentage of fish	70°C	90°C	120°C
Ragi	Control	5.90 (0.03)	5.41 (0.07)	5.71 (0.02)
	10% Ribbonfish	7.51 (0.04)	7.80 (0.04)	7.58 (0.09)
	20% Ribbonfish	6.61 (0.06)	5.51 (0.06)	ND
	10% Bull’s eye fish	7.42 (0.06)	7.39 (0.03)	7.19 (0.05)
	20% Bull’s eye fish	5.92 (0.05)	6.45 (0.02)	ND

C

Flour	Percentage of fish	70°C	90°C	120°C
Ragi	Control	11.85 (0.15)	11.12 (0.05)	11.58 (0.19)
	10% Ribbonfish	13.36 (0.13)	13.77 (0.03)	13.85 (0.17)
	20% Ribbonfish	12.74 (0.24)	11.77 (0.21)	ND
	10% Bull’s eye fish	13.22 (0.15)	13.23 (0.17)	13.21 (0.07)
	20% Bull’s eye fish	11.14 (0.20)	12.63 (0.03)	ND

ND: Not determined; Values in parenthesis represent standard deviation
(Products prepared at a constant screw speed of 350 RPM using a die diameter of 4 mm)

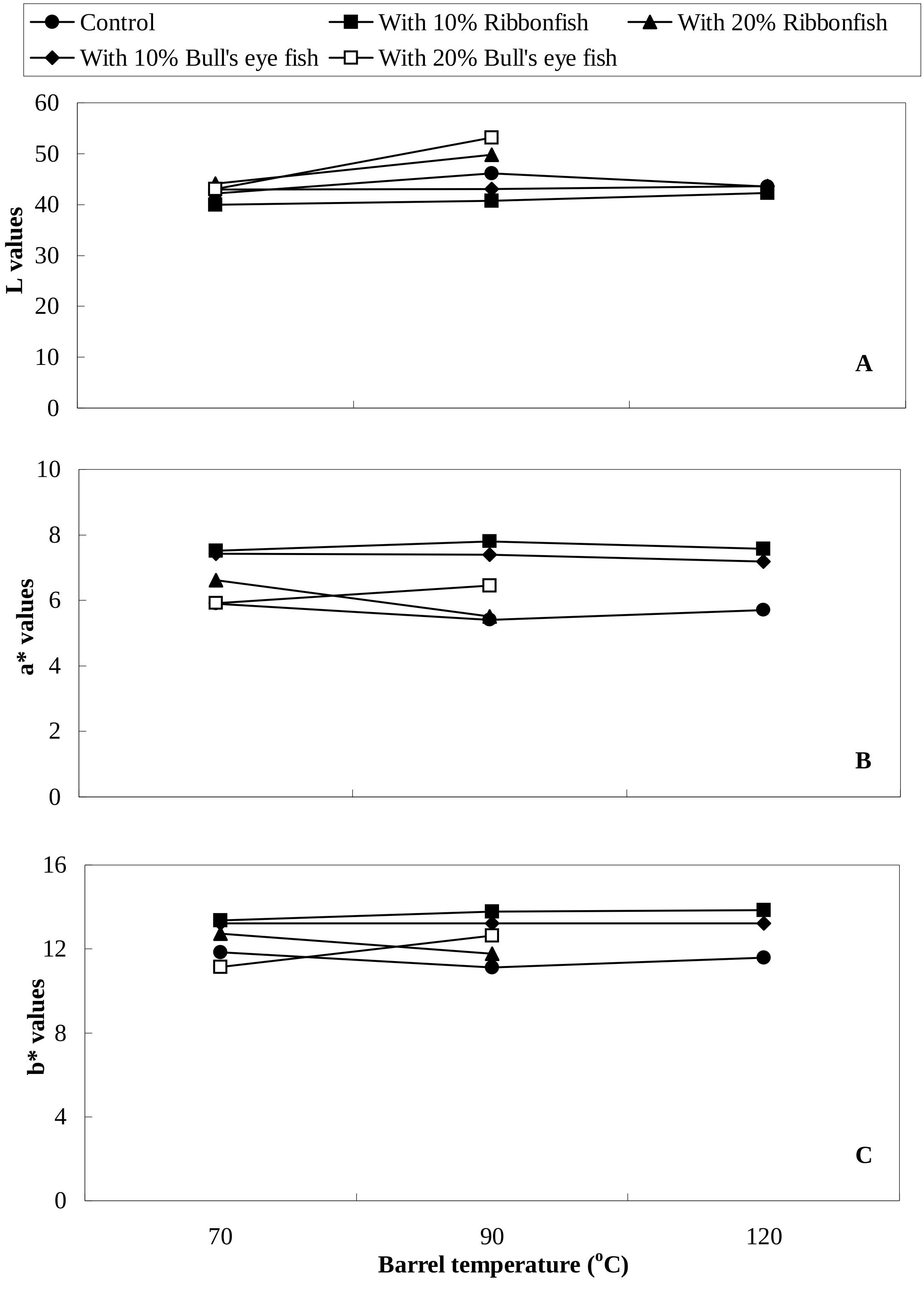

Fig. 38: Color values of extruded ragi flour-fish mince mixture as a function of barrel temperature A: L* values; B: a* values; C: b* values (Products were prepared at a constant screw speed of 350 RPM using a 4 mm die diameter

Table 25 A. F- ratios from results of Analysis of Variance of color values of rice flour based products as a function of barrel temperature

Sources of variation	L value		a* value		b* value	
	RF	BE	RF	BE	RF	BE
Between Temperature	0.10	0.52	0.92	2.24	0.57	1.12
Between samples	0.15	0.01	0.04	0.13	2.59	10.97

Statistical analysis has been carried out between control samples and products with 10% fish meat only. (RF: Ribbonfish mince; BE: Bull's eye fish mince)
No significant difference between samples as a function of barrel temperature ($P<0.05$)

Table 25 B. F-ratios from results of Analysis of Variance of color values of wheat semolina based products as a function of barrel temperature

Sources of variation	L value		a* value		b* value	
	RF	BE	RF	BE	RF	BE
Between Temperature	1.66	6.72	0.21	13.06	1.29	0.44
Between samples	0.42	33.65*	3.77	80.80*	0.15	0.04

Statistical analysis has been carried out between control samples and products with 10% fish meat only. (RF: Ribbonfish mince; BE: Bull's eye fish mince)
* Denotes significant difference ($P<0.05$)

Table 25 C. F-ratios from results of Analysis of Variance of color values of ragi flour based products as a function of barrel temperature

Sources of variation	L value		a* value		b* value	
	RF	BE	RF	BE	RF	BE
Between Temperature	1.33	0.94	0.06	0.98	0.22	0.93
Between samples	5.83	0.39	72.81*	107.08*	40.90*	61.76*

Statistical analysis has been carried out between control samples and products with 10% fish meat only. (RF: Ribbonfish mince; BE: Bull's eye fish mince)
* Denotes significant difference ($P<0.05$)

4.8.5.2. As a function of screw speed

The L values of extruded products from mixture of different flours (rice flour, wheat semolina and ragi flour) and fish mince as a function of screw speed is given in Table 26 A, Fig. 39. The L value of all the samples increased with increase in screw speed except the product with 10% ribbonfish, where the L value decreased with screw speed. Only with the product with 10% ribbonfish mince L values was higher than that of the control. The L values of products from ragi flour were comparatively lower than that of rice flour and wheat semolina based extruded product. Extruded rice flour based samples showed significant ($P<0.05$) increase in L values with the addition of 10% ribbonfish, while addition of 10% ribbonfish decreased the L values significantly ($P<0.05$) in ragi flour based products (Table 26 B).

The a* values of extruded products from different flours/semolina prepared as a function of screw speed is given in Table 27 A, Fig. 40. The a* values of rice flour and wheat semolina based products decreased with increase in screw speed, whereas ragi based products showed an opposite trend. The decrease in a* values for wheat semolina based extruded products was significant at 5% level of significance (Table 27 B). The products which were having higher L values showed lower a* values. In case of wheat semolina and ragi flour based products, those with fish mince had higher a* value than the control samples.

The b* values of extruded products from different flour materials with 10% ribbonfish mince prepared as function of screw speed is given in Table 28 A, Fig. 41. Extruded rice based products showed a decreasing trend in b* values with screw speed. In the case of wheat semolina based products b* value increased when the screw speed increased from 300 RPM to 400 RPM and then it decreased at screw speed of 500 RPM. In the case of ragi based extruded products the b* values showed an increasing trend with increase in screw speed. Addition of 10% ribbonfish mince caused significant ($P<0.05$) increase in b* values of rice flour/wheat semolina and ragi flour based extruded products (Table 28 B).

Table 26 A. L* values of extruded products* from flour- fish mince mixture as a function of screw speed

Raw material	Screw speed		
	300 RPM	400 RPM	500 RPM
Rice Control	62.75 (0.52)	63.97 (0.39)	64.39 (1.39)
Rice + 10% Ribbonfish	80.87 (0.27)	78.69 (0.67)	75.75 (0.39)
Wheat Control	63.04 (0.57)	64.52 (0.20)	67.42 (0.10)
Wheat + 10% Ribbonfish	57.56 (0.15)	63.82 (0.71)	69.35 (1.34)
Ragi Control	45.37 (0.30)	46.49 (0.10)	53.07 (0.15)
Ragi + 10% Ribbonfish	35.06 (0.42)	39.85 (0.19)	39.52 (0.10)

(*Products prepared at a barrel temperature of 90^{o}C using a 4 mm die diameter)

Table 26 B. F-ratios from results of Analysis of Variance of L values of products as a function of screw speed

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Screw speed	0.28	4.63	3.09
Between samples	57*	0.43	25.94*

RF: Products with ribbonfish mince

* Denotes significant difference (P<0.05)

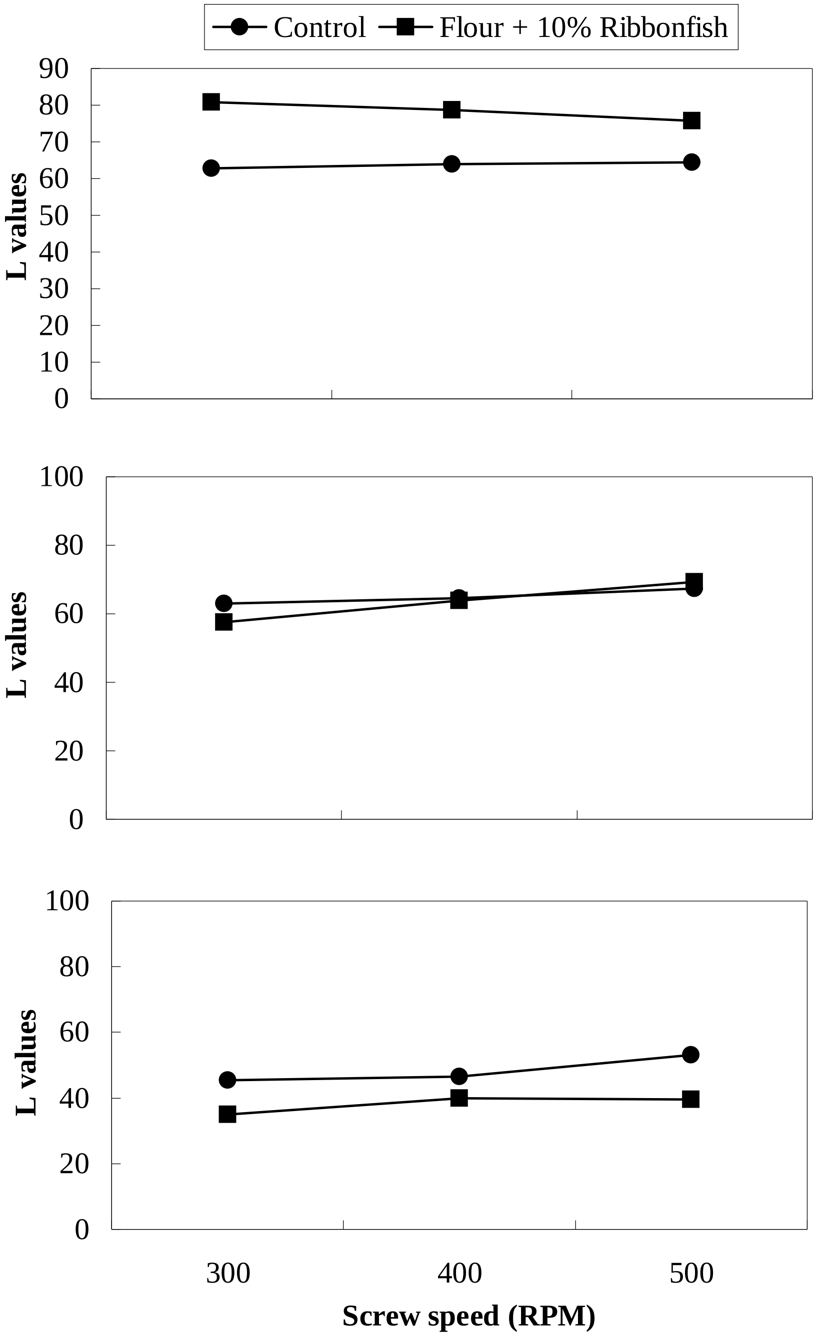

Fig. 39: L values of flour-fish mince mixture extruded as a function of screw speed (Products were prepared at a constant barrel temperature of 90^{o}C using a 4 mm die diameter)

Table 27 A. a* values of extruded products from flour-fish mince mixture as a function of screw speed

Raw material	Screw speed		
	300 RPM	400 RPM	500 RPM
Rice Control	1.76 (0.04)	1.37 (0.03)	0.75 (0.03)
Rice+10% Ribbonfish	0.23 (0.05)	0.20 (0.01)	0.09 (0.03)
Wheat Control	1.45 (0.04)	0.80 (0.01)	0.70 (0.04)
Wheat+10% Ribbonfish	2.18 (0.02)	1.58 (0.06)	1.38 (0.05)
Ragi Control	2.13 (0.01)	5.94 (0.03)	5.69 (0.08)
Ragi+10% Ribbonfish	6.59 (0.03)	7.96 (0.13)	7.51 (0.05)

(*Products prepared at a barrel temperature of 90°C using a 4 mm die diameter)

Table 27 B. F-ratios from results of Analysis of Variance of a* values of products as a function of screw speed

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Screw speed	1.77	270.33*	3.66
Between samples	19.69*	639.48*	10.63

RF: Products with ribbonfish mince

* Denotes significant difference ($P<0.05$)

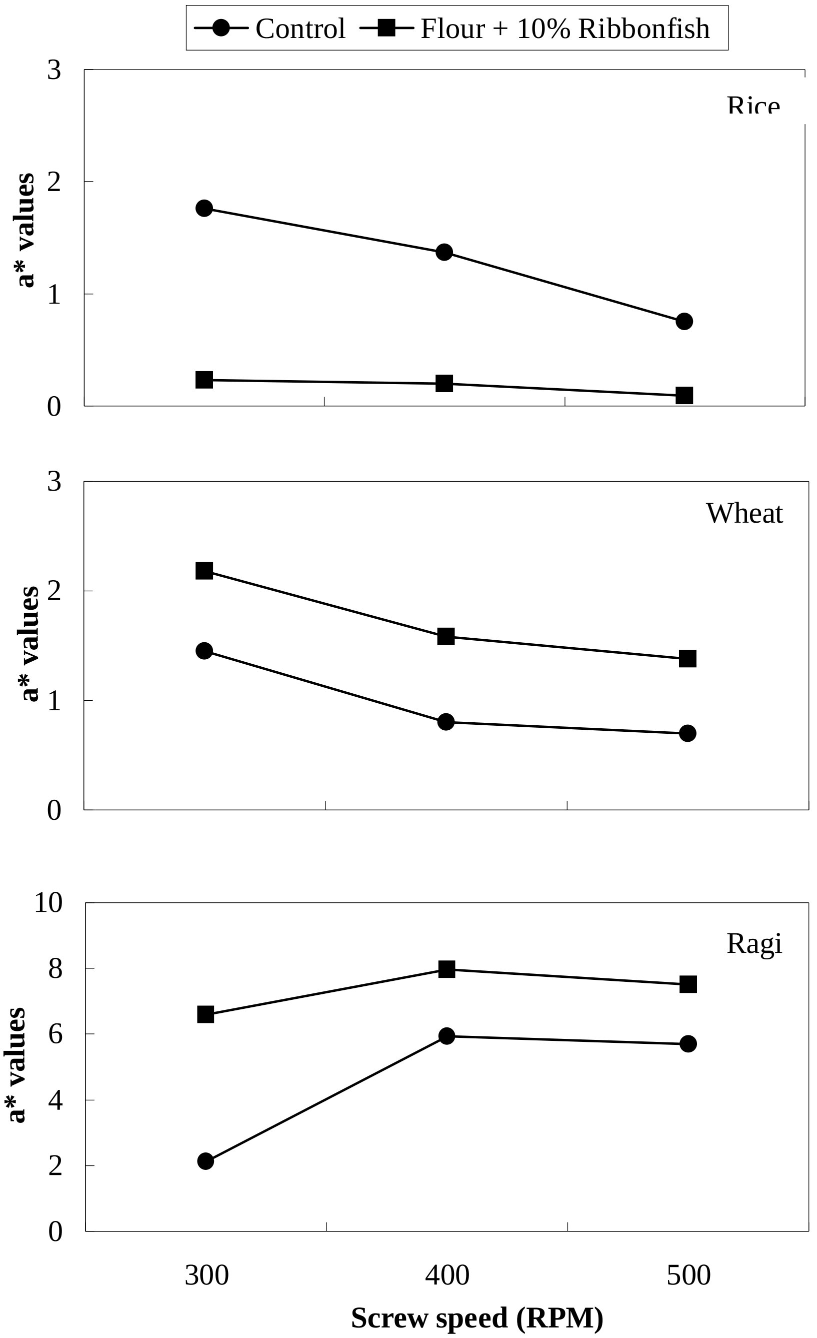

Fig. 40: a* values of flour-fish mince mixture as a function of screw speed (Products were prepared at a constant barrel temperature of 90°C using a 4 mm die diameter)

Table 28 A. b* values of extruded products from flour-fish mince mixture as a function of screw speed

Raw material	Screw speed		
	300 RPM	400 RPM	500 RPM
Rice Control	11.07 (0.17)	10.75 (0.29)	10.17 (0.17)
Rice+10% Ribbonfish	13.39 (0.08)	12.35 (0.22)	12.01 (0.12)
Wheat Control	14.30 (0.10)	15.13 (0.04)	13.77 (0.12)
Wheat+10% Ribbonfish	15.68 (0.03)	17.85 (0.56)	16.15 (0.09)
Ragi Control	10.01 (0.16)	12.02 (0.09)	12.15 (0.10)
Ragi+10% Ribbonfish	11.67 (0.13)	13.11 (0.08)	13.63 (0.12)

(*Products prepared at a barrel temperature of 90°C using a 4 mm die diameter)

Table 28 B. F-ratios from results of Analysis of Variance of b* values of products as a function of screw speed

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Screw speed	9.79	6.31	57.19*
Between samples	82.29*	28.85*	70.25*

RF: Products with ribbonfish mince

* Denotes significant difference ($P<0.05$)

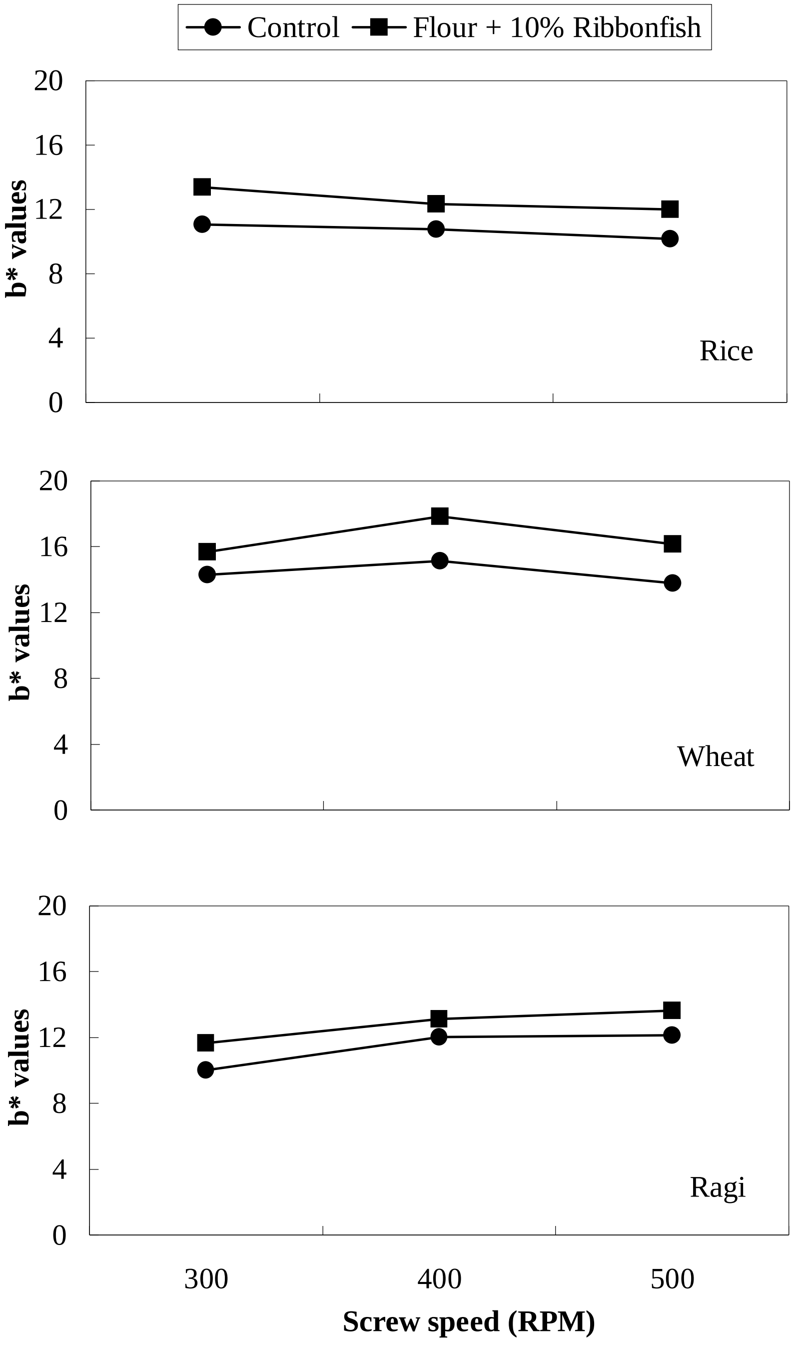

Fig. 41: b* values of flour-fish mince mixture extruded as a function of screw speed (Products were prepared at a constant barrel temperature of 90°C using a 4 mm die diameter)

4.8.5.3. As a function of die diameter

The L values of extruded products from mixture of different flours (rice flour, wheat semolina and ragi flour) and fish mince as a function of die diameter is given in Table 29 A, Fig. 42. The L values for all the samples showed an increasing trend with increase in die diameter except rice flour based samples, where the L value decreased when the die diameter increased from 4 mm to 5 mm. There was significant reduction in L value in wheat semolina based extruded products with the addition of 10% ribbonfish mince (Table 29 B). The a* values of different extruded products prepared as a function of die diameter is given in Table 30 A, Fig. 43. In the case of rice flour and wheat semolina based extruded products the trend of a* values was exactly opposite to that of L values, whereas the a* values of ragi based products showed significant ($P<0.05$) increase with increase in die diameter (Table 30 B). The b* values of extruded products prepared as function of die diameter is given in Table 31 A, Fig. 44. In case of rice based products, the product with fish mince showed an increasing b* value when die diameter increased from 3 mm to 4 mm and further it reduced when die diameter was 5 mm. The control samples showed a decreasing trend in b* values. Both control and sample showed a decreasing trend of b* values with an increase in die diameter in the case of wheat semolina based extruded products. In case of ragi flour based products the b* values increased significantly ($P<0.05$) with increase in die diameter (Table 31 B).

4.8.6. Texture analysis

4.8.6.1. Crispness

The results of texture analysis of extruded products from rice flour, wheat semolina and ragi flour is given in Fig. 45-50. Results showed that with an increase in process temperature the crispness of the product also increases. The number of major peaks is considered as an indication of crispiness. The products prepared with 10% fish mince at 90^{o}C appeared to be crispier irrespective of the flour or the fish used. Among the products prepared by the addition of 20% fish mince one, which was processed at 90^{o}C, appeared to be better. The number of peaks was maximum for the products prepared with 10% ribbonfish mince and processed at 90^{o}C. Extruded products from wheat semolina with 20% bull's eye fish and processed at 70^{o}C appears to be the tough. The number of peaks is less in the case of ragi-based products irrespective of the fish mince used compared to rice based products.

Table 29 A. L* values of extruded products from flour-fish mince mixture as a function of die diameter

Raw material	Die diameter		
	3 mm	4 mm	5 mm
Rice Control	68.14 (2.19)	73.26 (0.32)	62.87 (0.27)
Rice+10% Ribbonfish	61.05 (0.47)	76.58 (1.52)	68.74 (0.08)
Wheat Control	69.19 (2.05)	72.73 (0.12)	73.60 (0.21)
Wheat+10% Ribbonfish	59.46 (0.67)	66.62 (0.98)	67.13 (0.49)
Ragi Control	39.72 (0.30)	46.10 (0.32)	49.11 (0.07)
Ragi+10% Ribbonfish	39.83 (0.05)	41.65 (0.39)	42.25 (0.09)

(*Products prepared at a barrel temperature of 90°C and screw speed of 350 RPM)

Table 29 B. F-ratios from results of Analysis of Variance of L values of products as a function of die diameter

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Screw speed	2.70	10.99	2.92
Between samples	0.03	41.71*	3.34

RF: Products with ribbonfish mince

* Denotes significant difference ($P<0.05$)

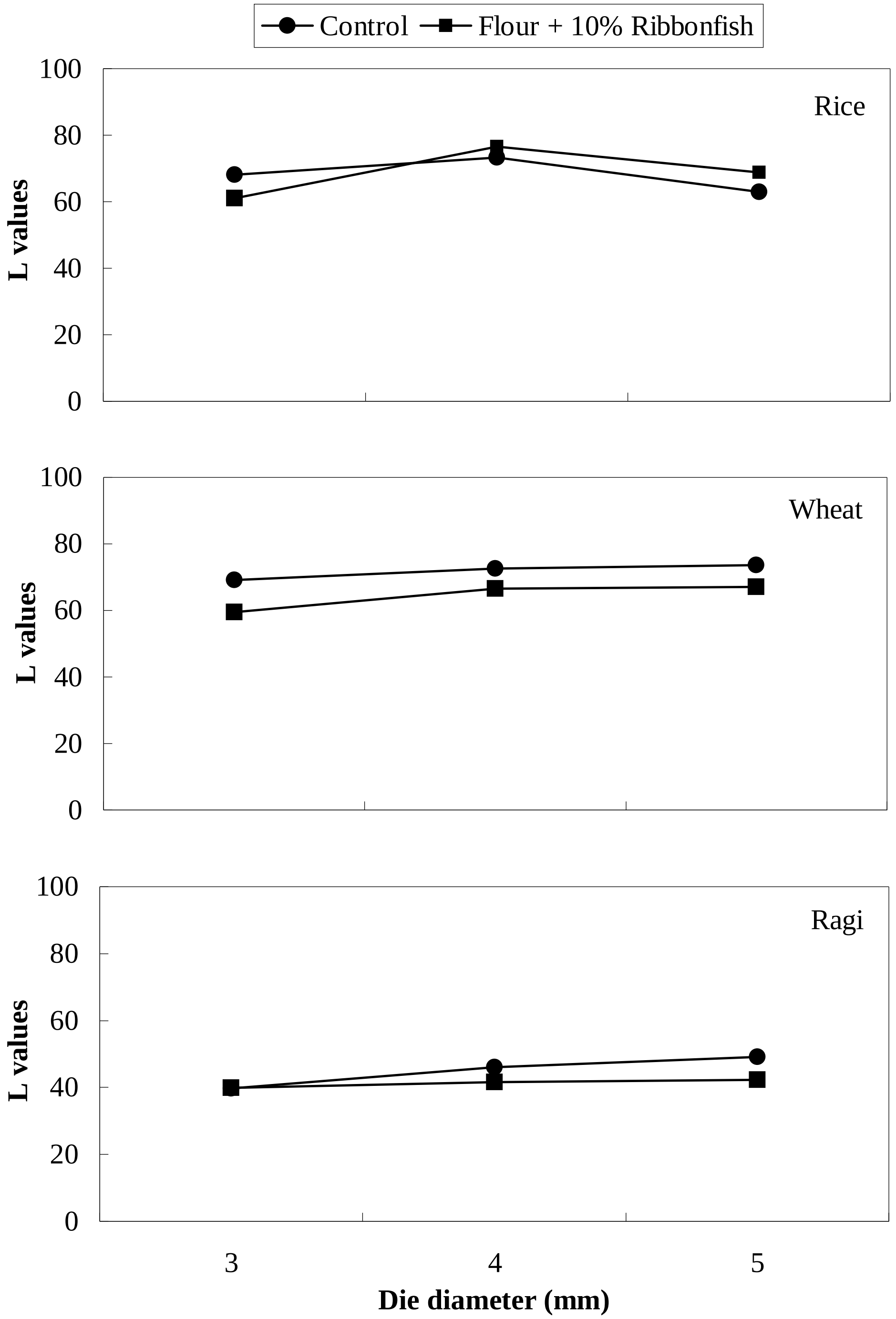

Fig. 42: L* values of extruded products from flour-ribbonfish mince mixture as a function of die diameter (Products prepared at a constant barrel temperature of 90°C and screw speed of 350 RPM)

Table 30 A. a* values of extruded products from flour-fish mince mixture as a function of die diameter

Raw material	Die diameter		
	3 mm	4 mm	5 mm
Rice Control	1.86 (0.04)	0.87 (0.02)	1.39 (0.03)
Rice+10% Ribbonfish	1.95 (0.05)	0.72 (0.01)	0.99 (0.01)
Wheat Control	1.34 (0.03)	1.24 (0.09)	0.93 (0.01)
Wheat+10% Ribbonfish	2.56 (0.05)	1.71 (0.02)	0.84 (0.03)
Ragi Control	4.54 (0.04)	5.41 (0.07)	6.83 (0.02)
Ragi+10% Ribbonfish	6.84 (0.09)	7.58 (0.09)	8.37 (0.17)

(*Products prepared at a barrel temperature of 90°C and screw speed of 350 RPM)

Table 30 B. F-ratios from results of Analysis of Variance of a* values of products as a function of die diameter

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Screw speed	21.09*	2.64	22.26*
Between samples	1.17	1.98	72.86*

RF: Products with ribbonfish mince
* Denotes significant difference ($P<0.05$)

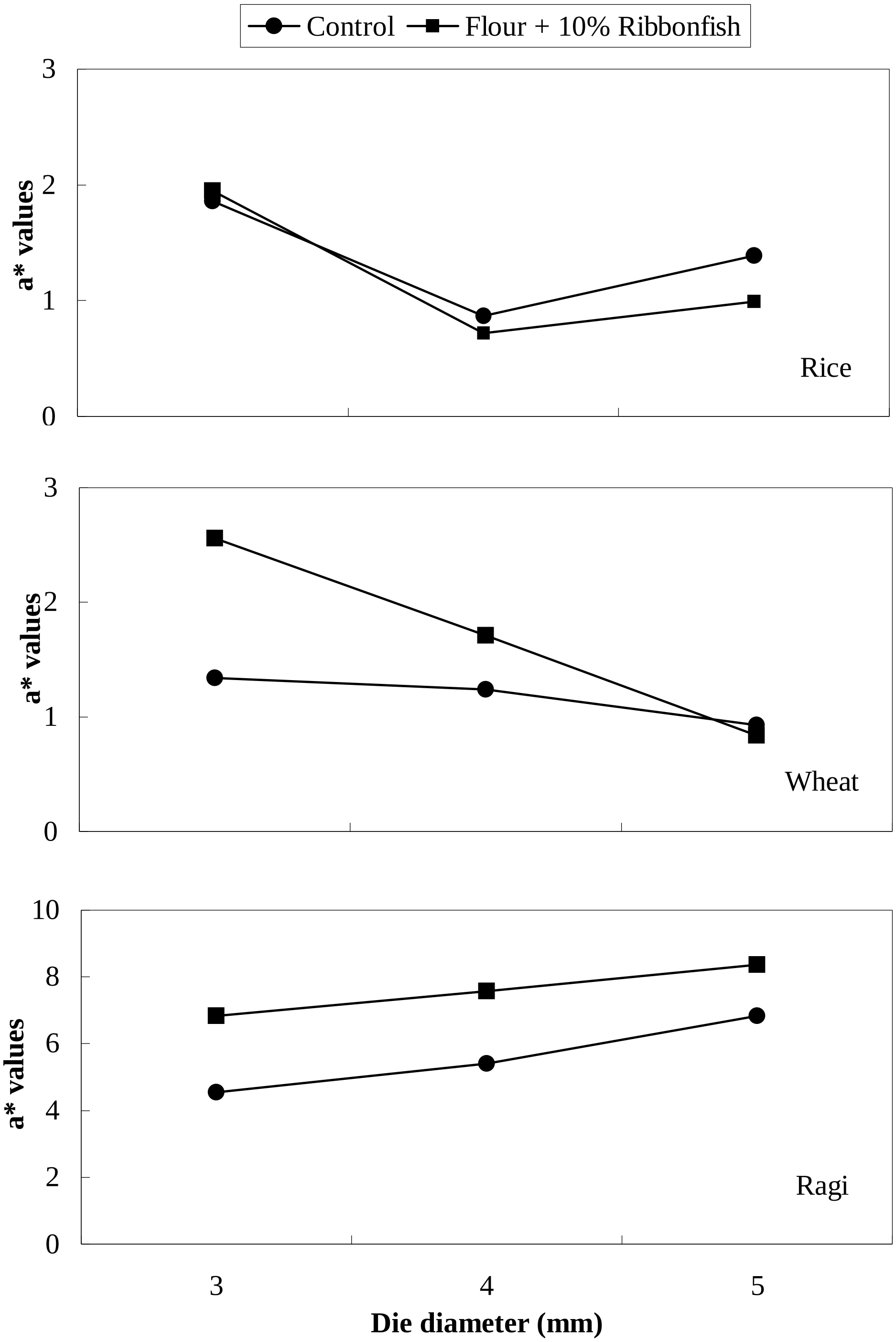

Fig. 43: a* values of extruded products from flour-ribbonfish mince mixture as a function of die diameter (Products prepared at a constant barrel temperature of 90°C and screw speed of 350 RPM)

Table 31 A. b* values of extruded products from flour-fish mince mixture as a function of die diameter

Raw material	Die diameter		
	3 mm	4 mm	5 mm
Rice Control	12.85 (0.21)	12.46 (0.05)	11.19 (0.12)
Rice+10% Ribbonfish	10.55 (0.04)	16.91 (0.16)	11.87 (0.02)
Wheat Control	16.34 (0.04)	15.35 (0.29)	13.76 (0.04)
Wheat+10% Ribbonfish	16.58 (0.19)	15.83 (0.10)	13.88 (0.06)
Ragi Control	9.61 (0.07)	11.12 (0.05)	13.42 (0.15)
Ragi+10% Ribbonfish	12.05 (0.07)	13.85 (0.17)	14.83 (0.14)

(*Products prepared at a barrel temperature of 90°C and screw speed of 350 RPM)

Table 31 B. F-ratios from results of Analysis of Variance of b* values of products as a function of die diameter

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Screw speed	1.10	215.46*	22.56*
Between samples	0.23	7.00	29.98*

RF: Products with ribbonfish mince

* Denotes significant difference ($P<0.05$)

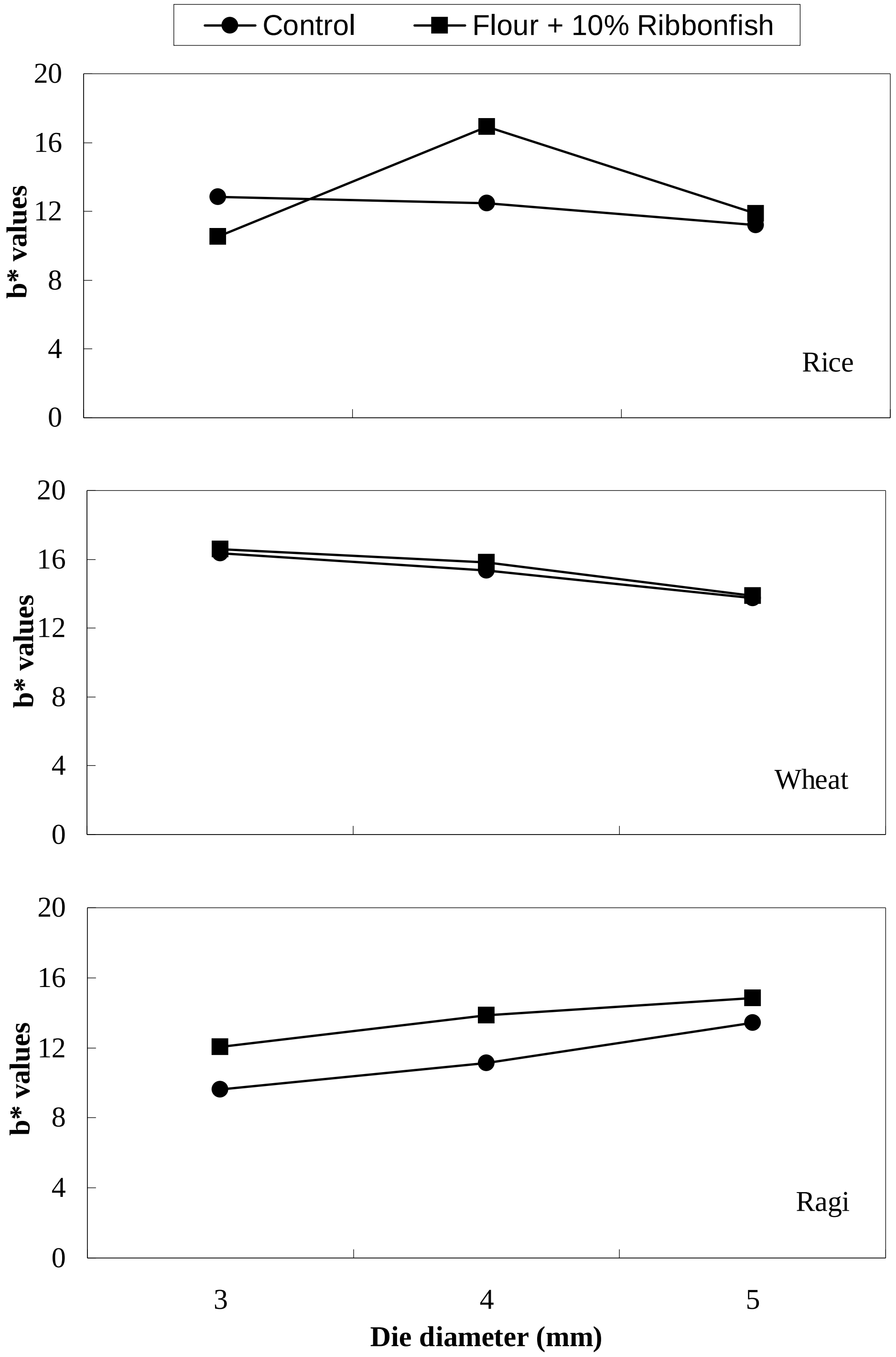

Fig. 44: b* values of extruded products from flour-ribbonfish mince mixture as a function of die diameter (Products prepared at a constant barrel temperature of 90°C and screw speed of 350 RPM)

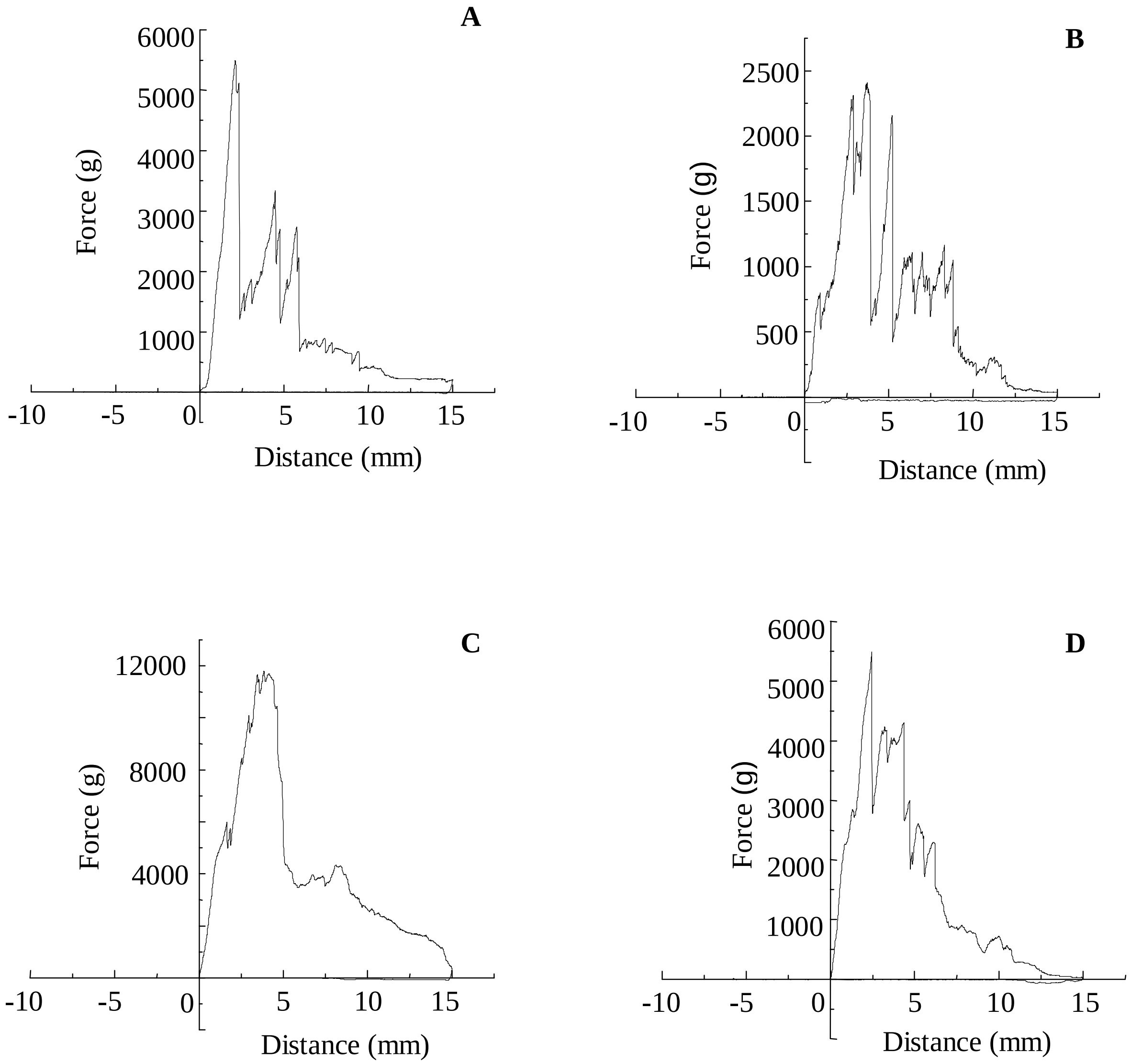

Fig. 45: Fracturability of extruded rice products with ribbonfish mince
A: 10% ribbonfish at 70°C B: 10% ribbonfish at 90°C
C: 20% ribbonfish at 70°C D: 20% ribbonfish at 90°C
(Products were prepared at a constant screw speed of 350 RPM using a 4 mm die diameter)

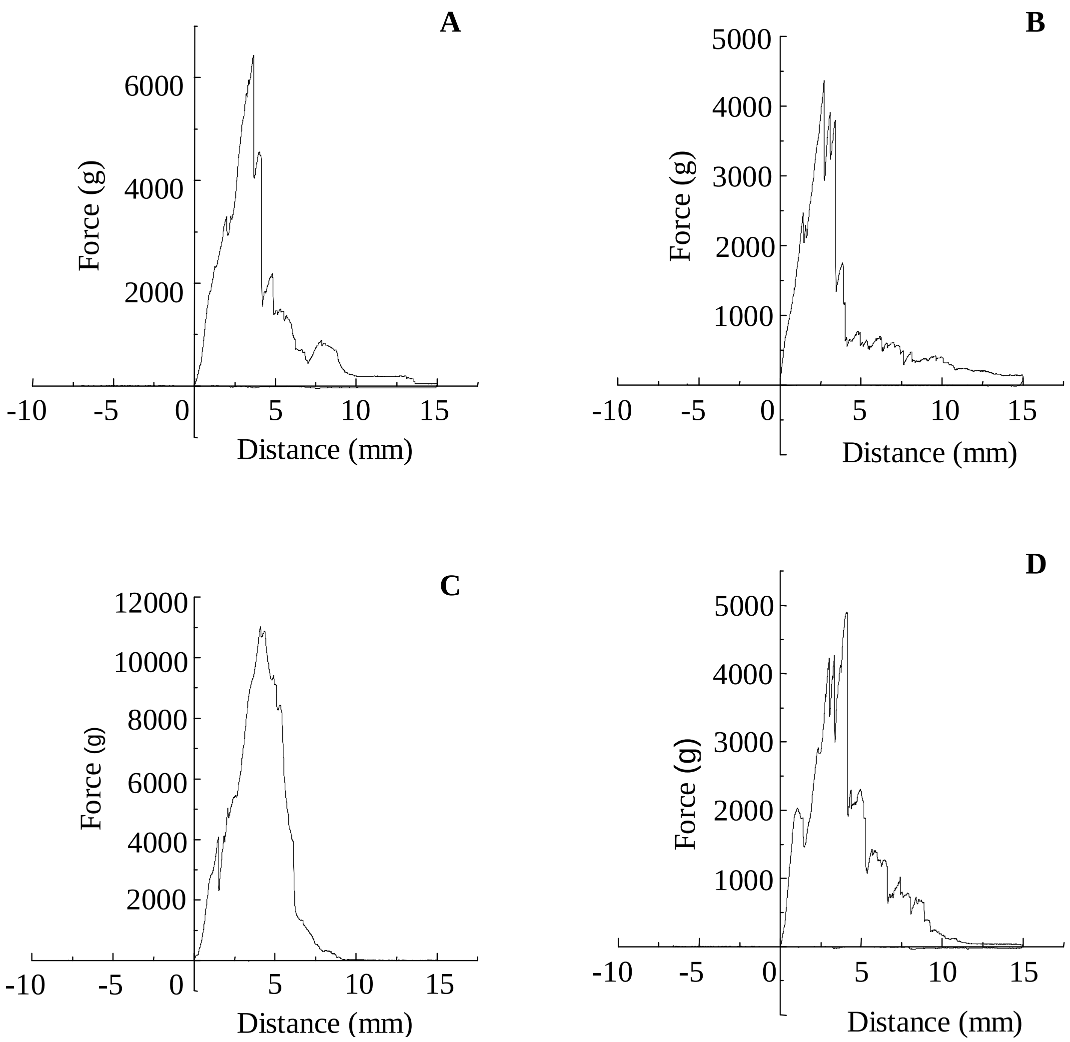

Fig. 46: Fracturability of extruded rice products with bull's eye fish mince
A: 10% bull's eye fish at 70^{o}C B: 10% bull's eye fish at 90^{o}C
C: 20% bull's eye fish at 70^{o}C D: 20% bull's eye fish at 90^{o}C
(Products were prepared at a constant screw speed of 350 RPM using a 4 mm die diameter)

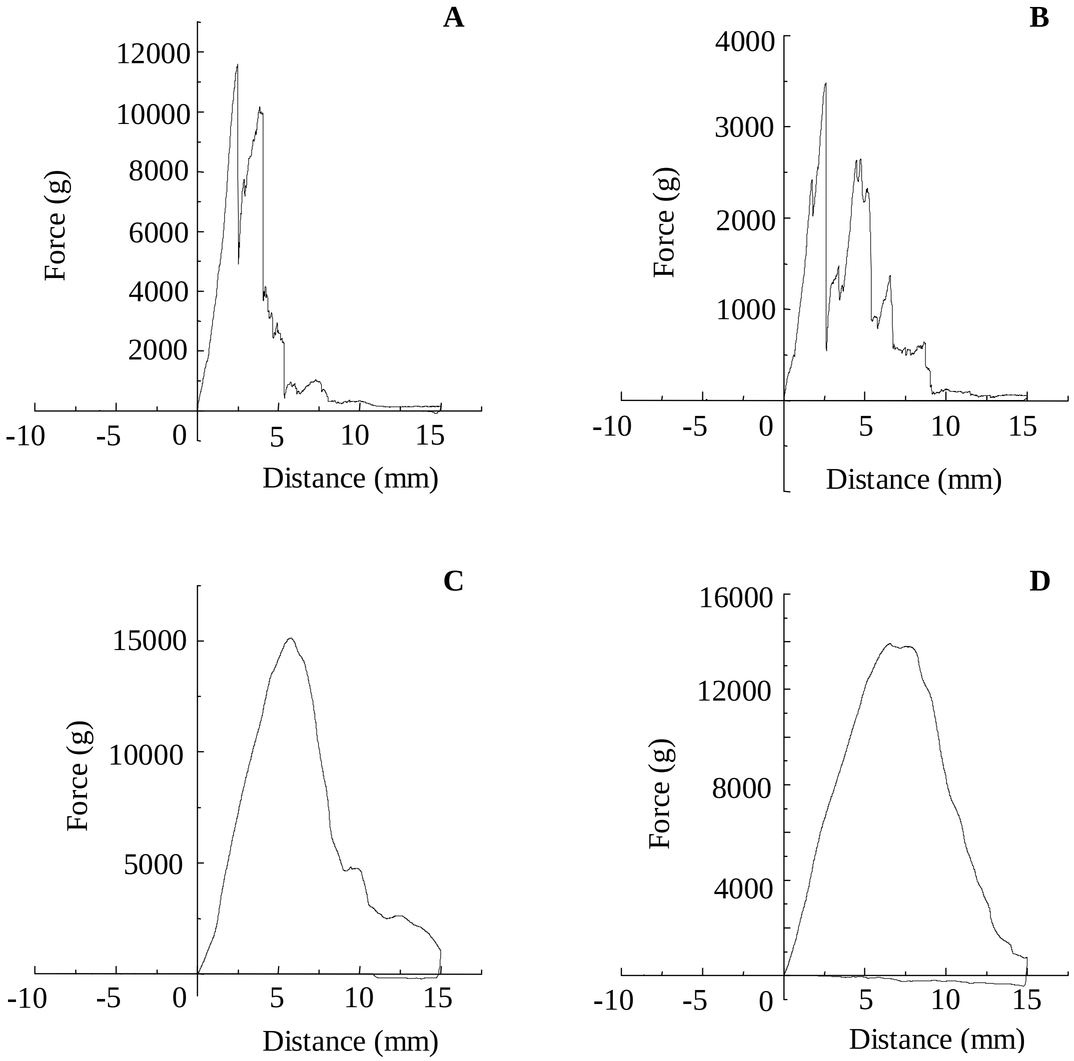

Fig. 47: Fracturability of extruded wheat semolina with ribbonfish mince
A: 10% ribbonfish at 70°C B: 10% ribbonfish at 90°C
C: 20% ribbonfish at 70°C D: 20% ribbonfish at 90°C
(Products were prepared at a constant screw speed of 350 RPM using a 4 mm die diameter)

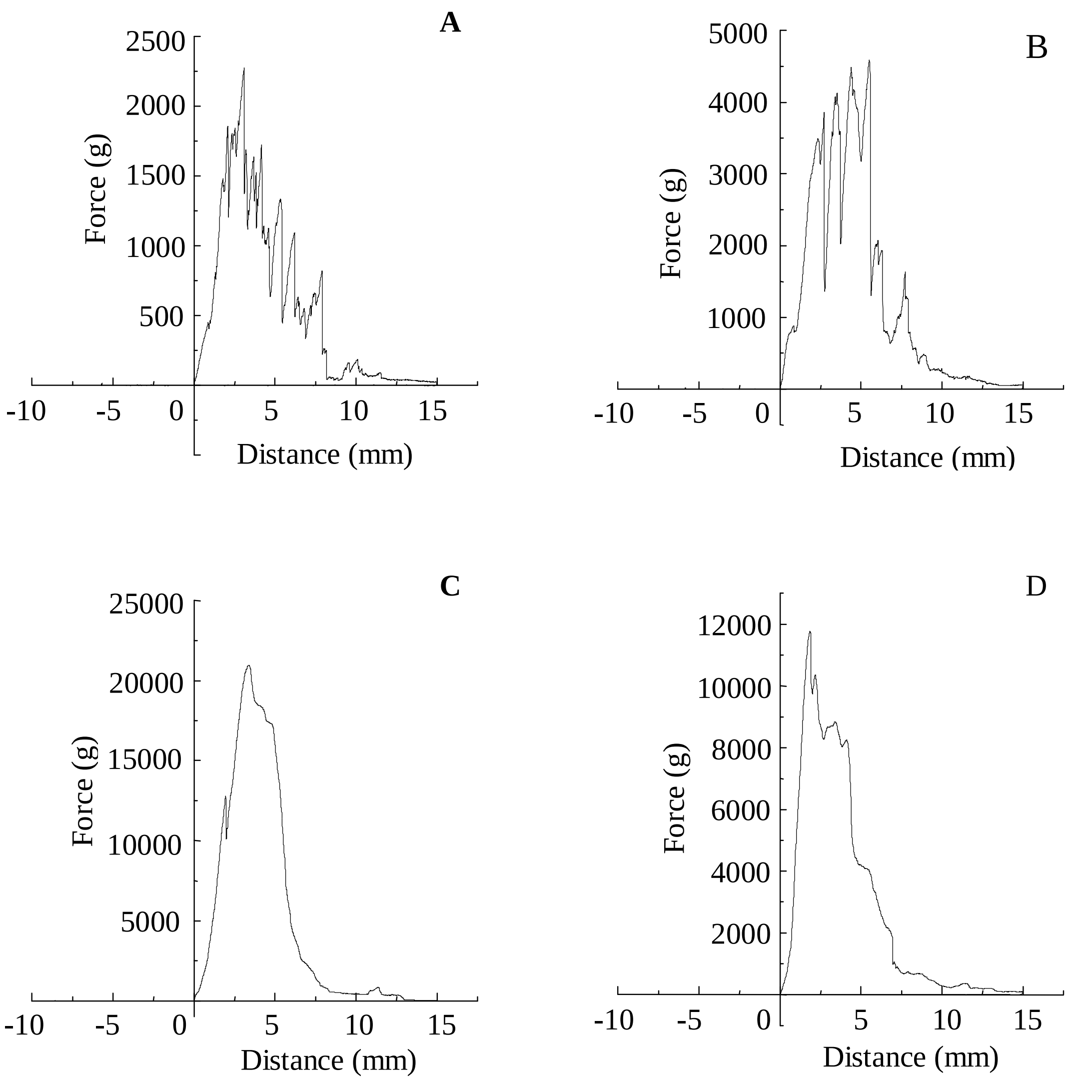

Fig. 48: Fracturability of extruded wheat semolina with bull's eye fish mince
A: 10% bull's eye fish at 70°C B: 10% bull's eye fish at 90°C
C: 20% bull's eye fish at 70°C D: 20% bull's eye fish at 90°C
(Products were prepared at a constant screw speed of 350 RPM using a 4 mm die diameter)

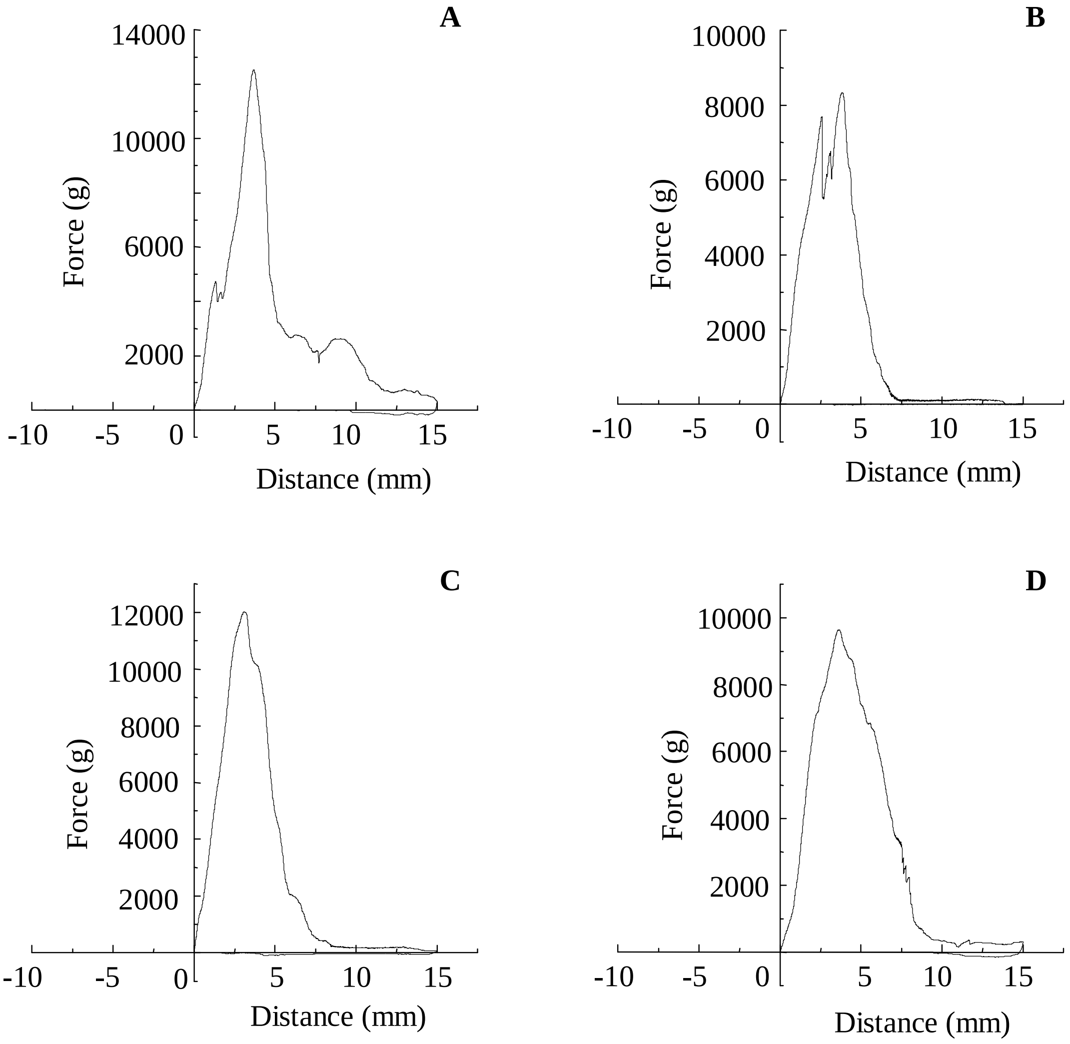

Fig. 49: Fracturability of extruded ragi flour with ribbonfish mince
A: 10% ribbonfish at 70°C B: 10% ribbonfish at 90°C
C: 20% ribbonfish at 70°C D: 20% ribbonfish at 90°C
(Products were prepared at a constant screw speed of 350 RPM using a 4 mm die diameter)

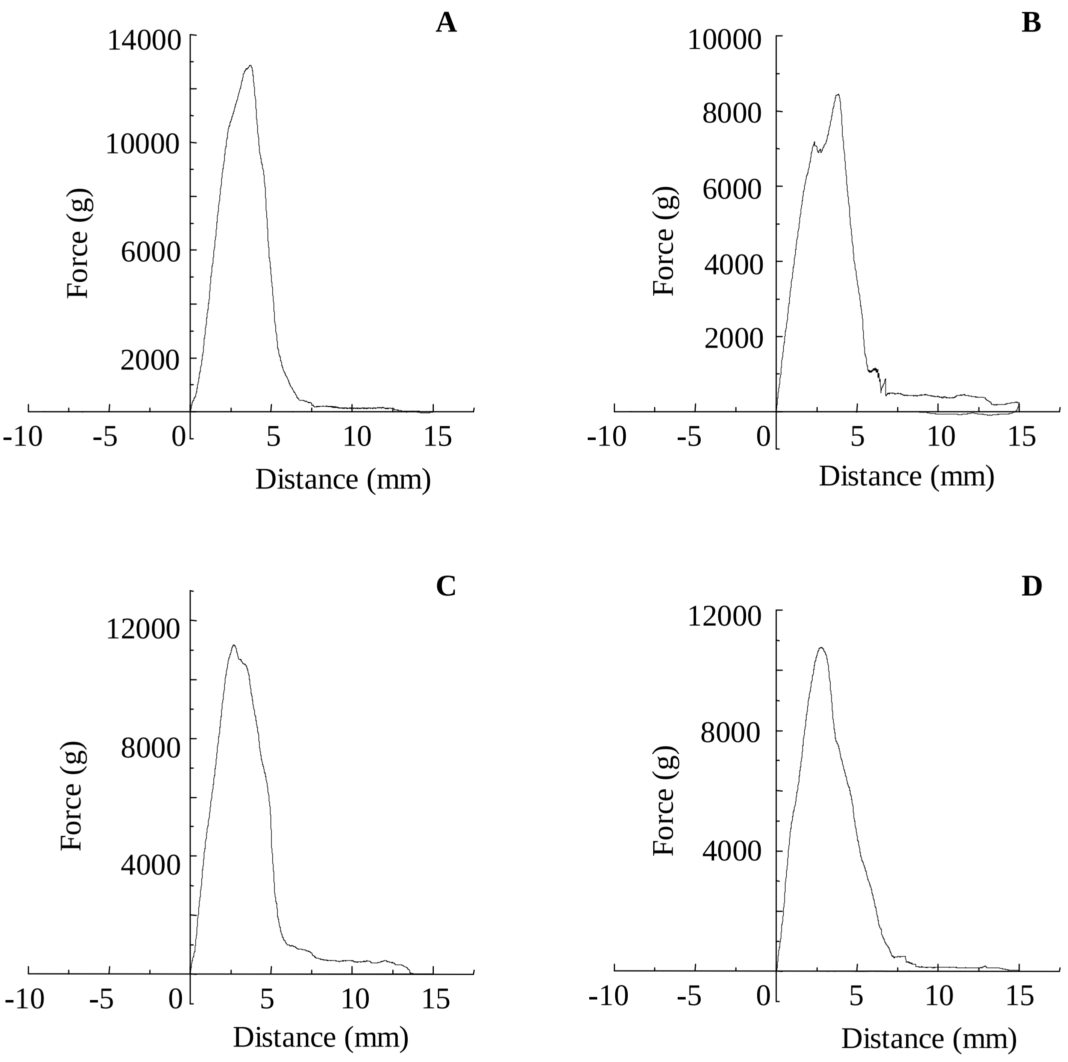

Fig. 50: Fracturability of extruded ragi flour with bull's eye fish mince
A: 10% bull's eye fish at 70^{o}C B: 10% bull's eye fish at 90^{o}C
C: 20% bull's eye fish at 70^{o}C D: 20% bull's eye fish at 90^{o}C
(Products were prepared at a constant screw speed of 350 RPM using a 4 mm die diameter)

4.8.6.2. Breaking strength

As a function of barrel temperature

Extruded products from mixture of various flour material (rice, wheat and ragi) with different percentage of fish mince prepared as a function of barrel temperature has been analyzed for their breaking strength and the results are given in Table 32 A, Fig. 51. In extruded rice based products with 10% of ribbonfish/bull's eye fish mince the breaking strength was much lower than the control. At 120°C all the three samples were having almost similar breaking strength. Breaking strength was highest (72.75 N) for products prepared with 20% ribbonfish mince at 70°C. With increase in barrel temperature the breaking strength decreased irrespective of the raw material used. These results were almost similar for wheat semolina based extruded products, but the absolute values of breaking strength recorded were high. The maximum force required was for the product with 20% ribbonfish mince with a force of 108.03 N. Breaking strength of extruded products from ragi flour revealed that the control sample was having the least value compared to that with fish mince content. Here also the highest value (83.96 N) was recorded for extruded products with 20% ribbonfish mince processed at 70°C. Breaking strength of ragi flour based extruded products increased significantly ($P<0.05$) with addition of 10% ribbonfish mince (Table 32 B). In all the cases the breaking strength increased with addition of 20% fish mince and decreased with increase in barrel temperature.

As a function of screw speed

Breaking strength of extruded products from mixture of various flour materials (rice, wheat and ragi) with 10% ribbonfish mince prepared as a function of screw speed at a constant barrel temperature of 90°C using a die diameter of 4 mm is given in Table 33 A, Fig. 52. In all the cases the breaking strength increased with increasing screw speed. Breaking strength of extruded rice and ragi flours with fish mince were below that of the control at any given screw speed, whereas the breaking strength of wheat control was significantly ($P<0.05$) higher than that of the product with 10% ribbonfish mince (Table 33 B). The force required was much higher for wheat semolina based extruded products when compared to that of the products from rice or ragi flour.

Table 32 A: Breaking strength of extruded products* from flour-fish mince mixture as a function of barrel temperature

Flour	Percentage of fish	Barrel temperature		
		70^{o}C	90^{o}C	120^{o}C
Rice control	Nil	33.42 (1.54)	22.23 (0.93)	12.19 (1.32)
Rice	10% Ribbonfish	24.61 (0.56)	20.56 (0.45)	13.45 (0.63)
	20% Ribbonfish	72.75 (2.91)	44.73 (3.72)	ND
	10% Bull's eye fish	21.17 (1.99)	18.48 (1.27)	11.02 (0.59)
	20% Bull's eye fish	57.53 (2.80)	25.32 (1.17)	ND
Wheat control	Nil	31.22 (0.99)	20.94 (0.78)	12.61 (1.07)
Wheat	10% Ribbonfish	20.55 (1.75)	16.46 (1.10)	11.76 (1.08)
	20% Ribbonfish	108.03 (0.37)	73.74 (4.99)	ND
	10% Bull's eye fish	20.16 (0.19)	14.83 (0.54)	8.62 (1.48)
	20% Bull's eye fish	65.11 (3.56)	31.58 (0.66)	ND
Ragi control	Nil	8.16 (1.19)	6.93 (0.81)	5.72 (0.56)
Ragi	10% Ribbonfish	30.13 (0.45)	27.23 (0.75)	23.67 (2.73)
	20% Ribbonfish	83.96 (0.39)	44.45 (1.33)	ND
	10% Bull's eye fish	23.50 (2.09)	11.21 (0.97)	9.55 (0.76)
	20% Bull's eye fish	58.39 (0.14)	49.26 (1.65)	ND

*Products were prepared at a constant screw speed of 350 RPM using a 4 mm die diameter
Values in Parenthesis denote standard deviation; (ND-Not determined)

Table 32 B: F-ratios from results of Analysis of Variance of breaking strength as a function of barrel temperature

Sources of variation	Rice flour		Wheat semolina		Ragi flour	
	RF	BE	RF	BE	RF	BE
Between Temperature	9.78	7.36	7.62	17.27	4.86	1.80
Between samples	1.06	2.92	3.46	11.33	296.37*	4.31

Statistical analysis has been carried out between control samples and products with 10% fish mince only. (RF: Ribbonfish mince; BE: Bull's eye fish mince)
* Denotes significant difference ($P<0.05$)

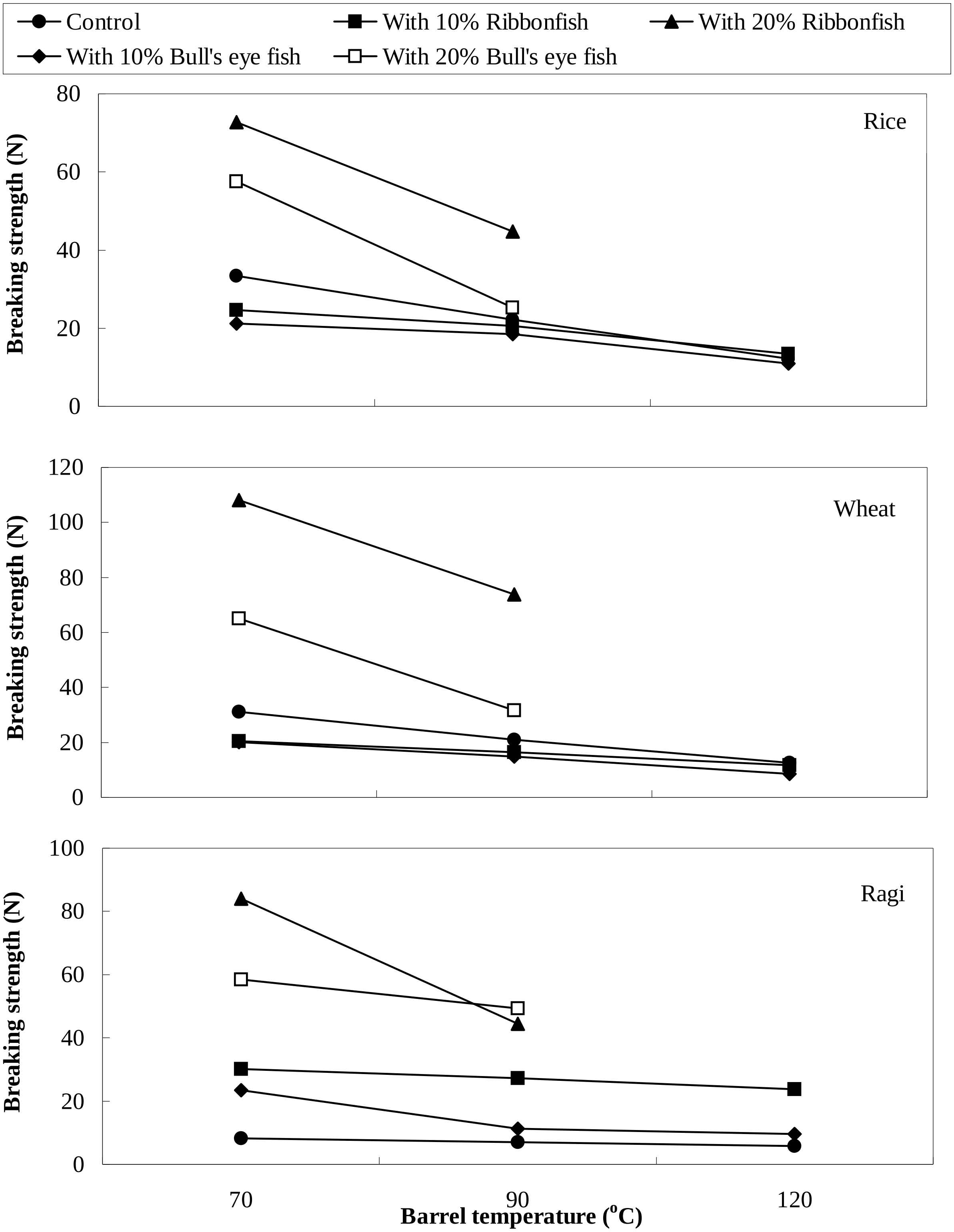

Fig. 51: Breaking strength of extruded products from flour-fish mince mixture as a function of barrel temperature (Products were prepared at a constant screw speed of 350 RPM using a 4 mm die diameter)

Table 33 A. Breaking strength of extruded products* from flour-fish mince mixture as a function of screw speed

Raw material	Screw speed		
	300 RPM	400 RPM	500 RPM
Rice Control	11.37 (1.31)	14.32 (0.06)	19.38 (2.27)
Rice+10% Ribbonfish	16.53 (2.35)	19.99 (1.99)	21.62 (1.78)
Wheat Control	32.85 (2.64)	33.27 (2.36)	39.35 (0.78)
Wheat+10% Ribbonfish	12.96 (0.60)	13.66 (0.87)	18.17 (0.71)
Ragi Control	8.59 (0.97)	9.07 (0.38)	9.09 (0.57)
Ragi+10% Ribbonfish	4.04 (0.45)	15.71 (0.04)	19.59 (0.31)

*Products were prepared at a constant barrel temperature of 90°C using a 4 mm die diameter

Table 33 B. F-ratios from results of Analysis of Variance of breaking strength as a function of screw speed

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Screw speed	12.52	59.54*	1.15
Between samples	16.62	1750*	0.86

RF: Products with ribbonfish mince

* Denotes significant difference ($P<0.05$)

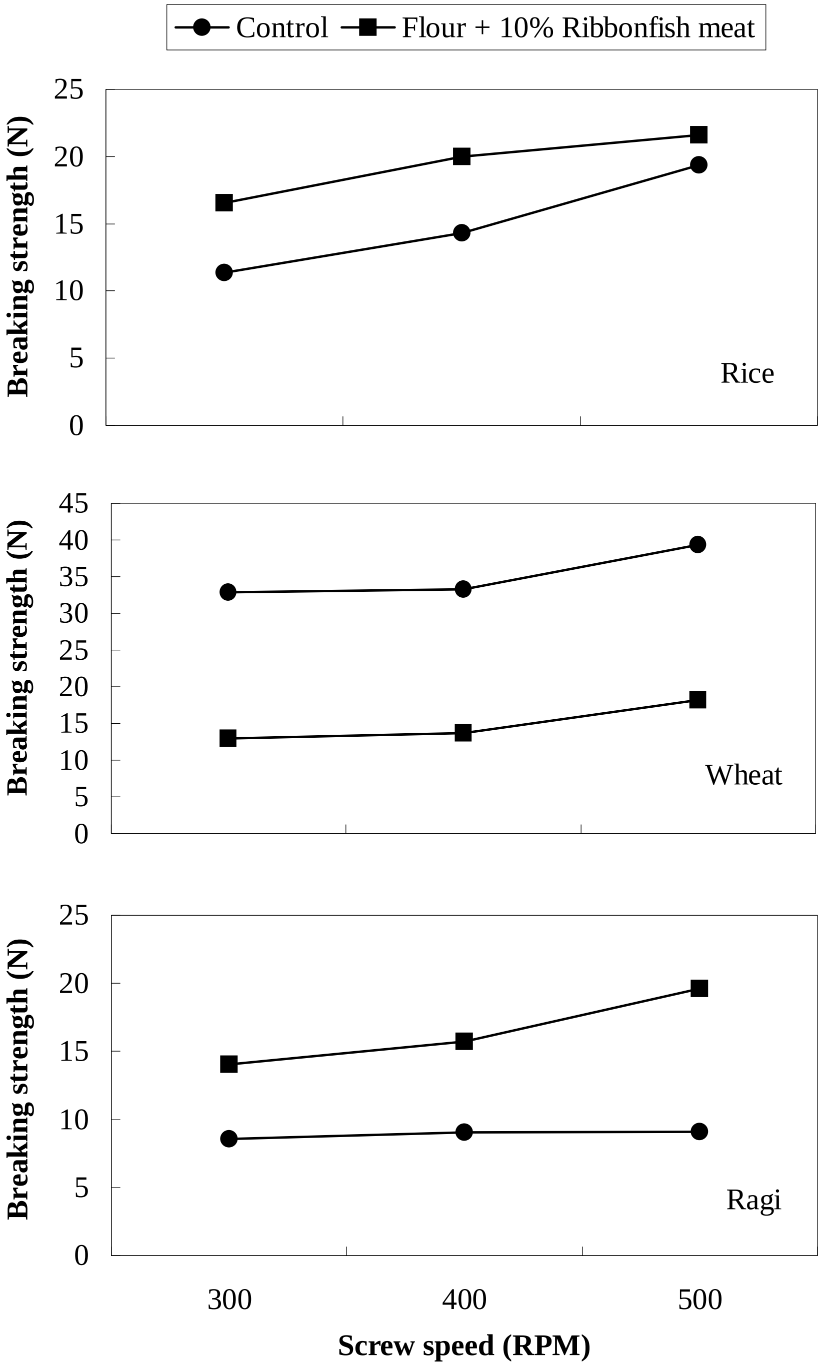

Fig. 52: Breaking strength of extruded products from flour-fish mince mixture as a function of screw speed (Products were prepared at a constant barrel temperature of 90°C using 4 mm die diameter)

As a function of die diameter

Breaking strength of extruded products from mixture of various flour materials (rice, wheat and ragi) with 10% ribbonfish mince prepared (barrel temperature- 90^{o}C; screw speed- 350 RPM) as a function of die diameter is given in Table 34 A, Fig. 53. Breaking strength of all the samples increased with increase in die diameter. However, the increase was significant with wheat semolina based extruded products (Table 34 B). Among the extruded products, the breaking strength for ragi flour alone extruded at 3 mm die diameter was lowest with a value of 4.96 N. A maximum value of breaking strength was recorded for rice flour samples when extruded through 5 mm die diameter (53.31 N). Breaking strength of extruded products with fish mince was found to be higher than that of the control in the case of rice and wheat semolina based products, while the reverse was found true in the case of ragi based products.

4.9. Gelatin

The gelatin was prepared using skin from bull's eye fish as detailed in section 3.2.2. The yield of the final gelatin was found to be 3.9%.

4.9.1. Proximate composition of bull's eye fish skin

The proximate composition of bull's eye fish skin is presented in Table 35. The moisture content of bull's eye fish skin was found to be 52.79% with a fat content of 1.24%. The protein content of the skin was 25.19% and the ash content was 20.23%.

4.9.2. Properties of freeze dried gelatin

The properties of freeze-dried gelatin are given in Table 36. The protein content was 94.6%, while the moisture content was 2.20%. The protein solubility of freeze-dried gelatin in buffer containing 0.3 M NaCl (pH: 7.5) was found to be 93% of total protein.

4.9.3. Aminoacid composition of gelatin

The aminoacid composition of gelatin from the skin of bull's eye fish is given in Table 37. The major aminoacid present in this gelatin was glycine (27.16). The total concentration of proline and hydroxyproline accounts to nearly 18% of total aminoacid.

Table 34 A. Breaking strength of extruded products* from flour-fish mince mixture as a function of die diameter

Raw material	Die diameter		
	3 mm	4 mm	5 mm
Rice Control	9.18 (0.71)	33.42 (1.54)	53.31 (2.01)
Rice+10% Ribbonfish	16.27 (0.79)	20.56 (0.97)	31.48 (1.32)
Wheat Control	14.52^{a} (0.49)	20.94^{b} (0.78)	41.54^{c} (0.81)
Wheat+10% Ribbonfish	12.84 (0.31)	16.46 (1.10)	36.04 (2.96)
Ragi Control	4.96 (0.70)	6.93 (0.81)	13.54 (2.76)
Ragi+10% Ribbonfish	12.21 (0.71)	26.32 (1.60)	27.23 (0.75)

*Products were prepared at a constant barrel temperature of 90°C and screw speed of 350 RPM

Table 34 B. F-ratios from results of Analysis of Variance of breaking strength as a function of die diameter

Sources of variation	Rice flour	Wheat semolina	Ragi flour
	RF	RF	RF
Between Die diameter	4.02	180.51*	3.93
Between samples	1.16	11.58	14.69

RF: Products with ribbonfish mince

* Denotes significant difference (P<0.05)

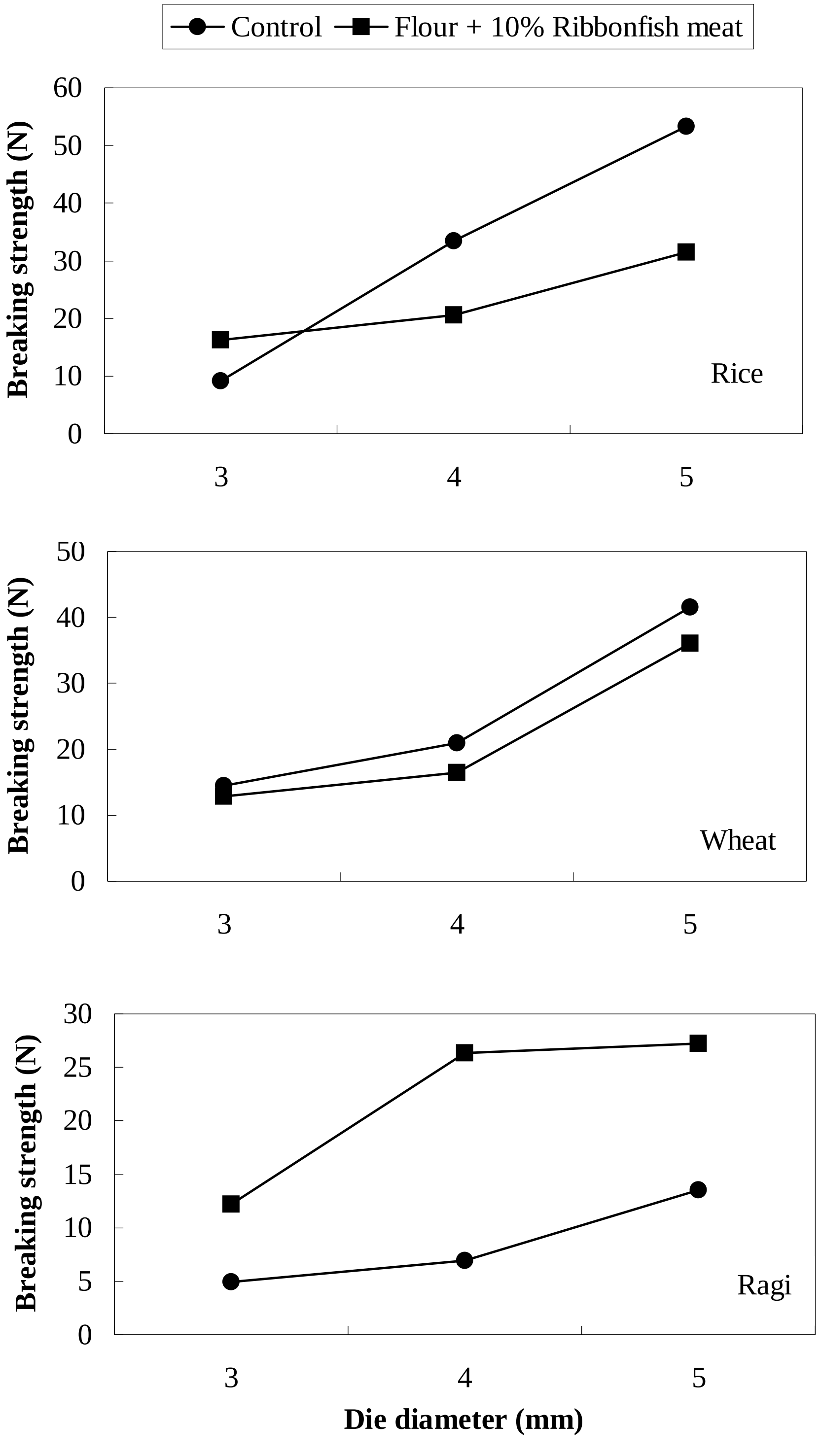

Fig. 53: Breaking strength of extruded products from flour-fish mince mixture as a function of die diameter (Products prepared at a constant barrel temperature of 90^{o}C and screw speed of 350 RPM)

Table 35. Proximate composition of skin of Bull's eye (*Priacanthus spp.*) fish

Parameters	Percentage
Moisture	52.79 (1.8)
Protein	25.19 (0.98)
Fat	1.24 (0.08)
Ash	20.23 (0.86)

Values in parenthesis are standard deviation, n = 3

Table 36. Properties of freeze dried gelatin

Protein content (N x 5.5) (%)	94.6 (1.2)
Moisture (%)	2.20 (0.05)
Solubility in 0.3 M NaCl (% of total protein)	93
Bloom strength (g)	108

Values in parenthesis represent standard deviation, n = 3

Table 37. Aminoacid composition of gelatin from skin of bull's eye fish (*Priacanthus spp.*)

Amino acids*	Mean values	Std Dev
Asp	0.98	0.12
Glu	1.47	0.19
H. Pro	13.45	0.24
Ser	2.31	0.33
Gly	27.16	0.68
His	2.16	0.50
Arg	2.60	0.83
Thr	3.08	0.41
Ala	19.45	0.82
Pro	4.98	1.64
Tyr	2.46	0.33
Val	2.18	0.22
Met	2.07	0.33
Cys	1.89	0.11
I Leu	1.25	0.09
Leu	2.78	0.07
Phe	3.36	0.60
Trp	2.48	3.08
Lys	3.21	0.83

*Expressed as % aminoacid

4.9.4. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis of gelatin

SDS-PAGE pattern of gelatin from skin of bull's eye fish showed two conspicuous bands at molecular weight range of 120 KD and 95 KD (Fig. 54).

4.9.5. Setting index of gelatin at different concentrations

Setting index showed the time taken for the gel to solidify completely at 5°C as a function of concentration. The data are represented in Table 38, Fig. 55. The data revealed that with increase in concentration of gelatin solution time taken for complete solidification decreased. At a concentration of 10 mg/ml, 100% solidification occurred after 180 minutes. When the concentration of the solution was increased to 40% time taken for complete solidification was 105 minutes.

4.9.6. Rheological properties of gelatin solution

The flow behavior of the gelatin solution as a function of gelatin concentration and temperature is given in Fig. 56-59. The flow behaviour of gelatin solution at a concentration of 10 mg/ml as a function of temperature is given in Fig. 56. At 40°C and 50°C the structural impairment of gelatin molecule was evident from the down curve data. A similar pattern was observed at a gelatin concentration of 15 mg/ml and 30 mg/ml (Fig. 57&58).

The flow behavior of gelatin solution (30 mg/ml) at 10°C revealed minimum thixotropic area (Fig. 59). When the shear rate decreased to log 1, the viscosity attained was log –1.12 Pa.s. With further reduction in shear rate the gelatin samples could not attain viscosity value equivalent to up curve data.

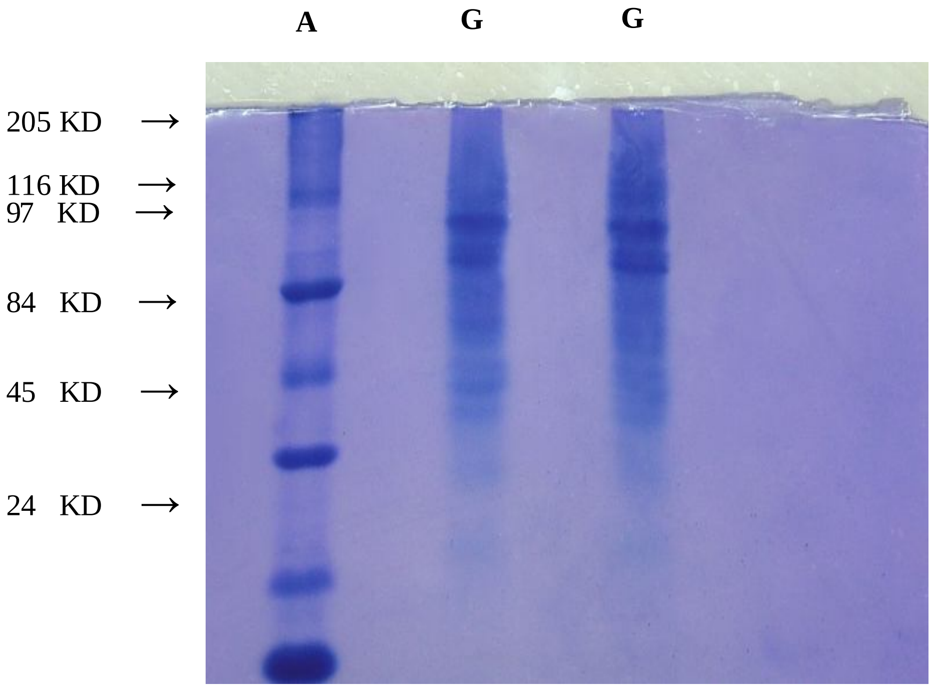

Fig. 54: SDS-PAGE pattern of gelatin from skin of bull's eye fish
S: Standard molecular marker G: Gelatin sample

Table 38. Setting index of gelatin at different concentrations

Time (min.)	10 mg/ml	20 mg/ml	30 mg/ml	40 mg/ml
15	0	0	0	0
30	0	0	5	10
45	0	5	10	20
60	5	10	25	40
75	10	25	40	60
90	20	40	60	75
105	30	50	80	100
120	40	60	95	100
135	60	70	100	100
150	70	95	100	100
165	90	100	100	100
180	100	100	100	100

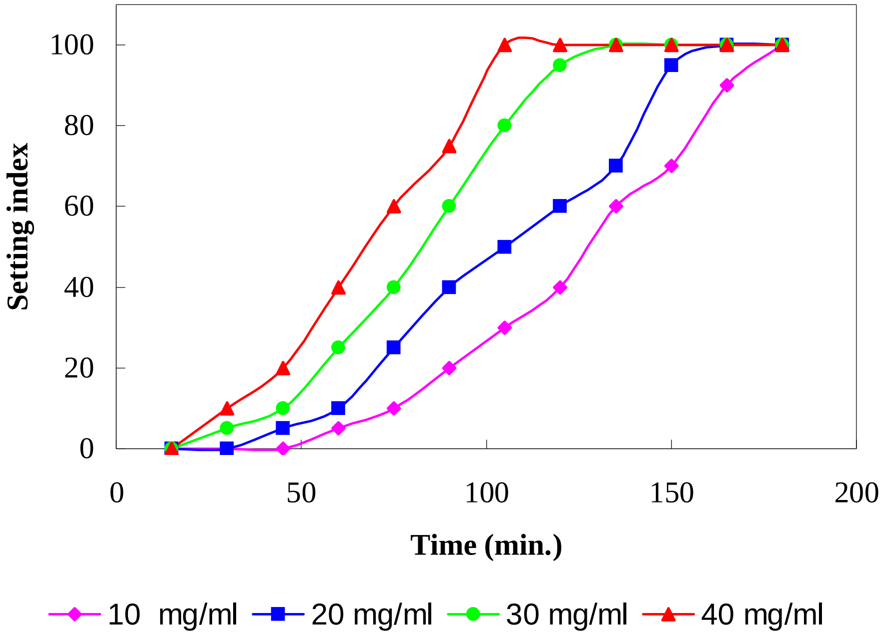

Fig. 55: Setting time of gelatin from skin of Bull's eye (*Priacanthus spp*) as a function of concentration

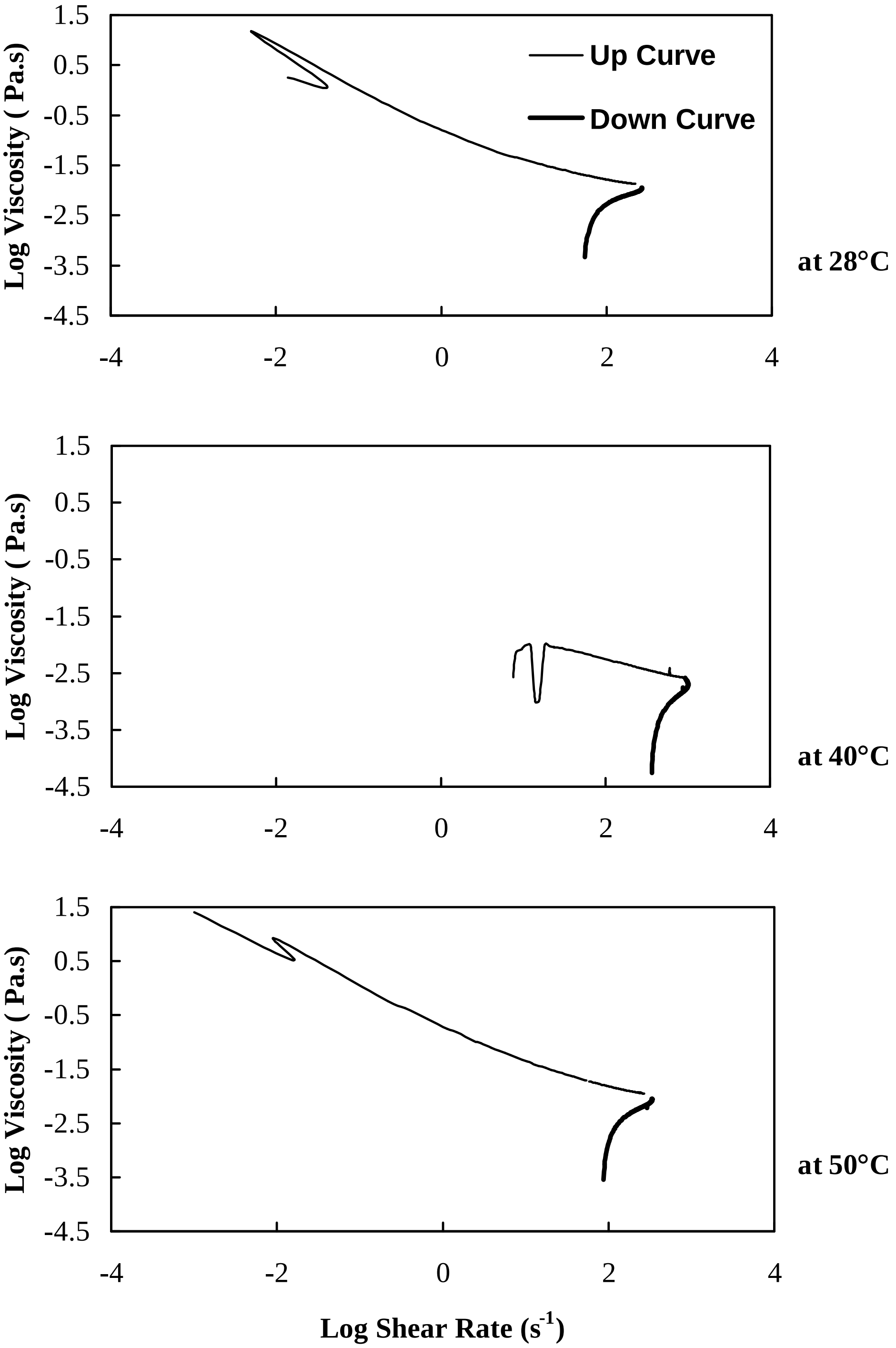

Fig. 56: Shear stress sweep of gelatin solution (10 mg/ml) from skin of Bull's eye (*Priacanthus spp.*)

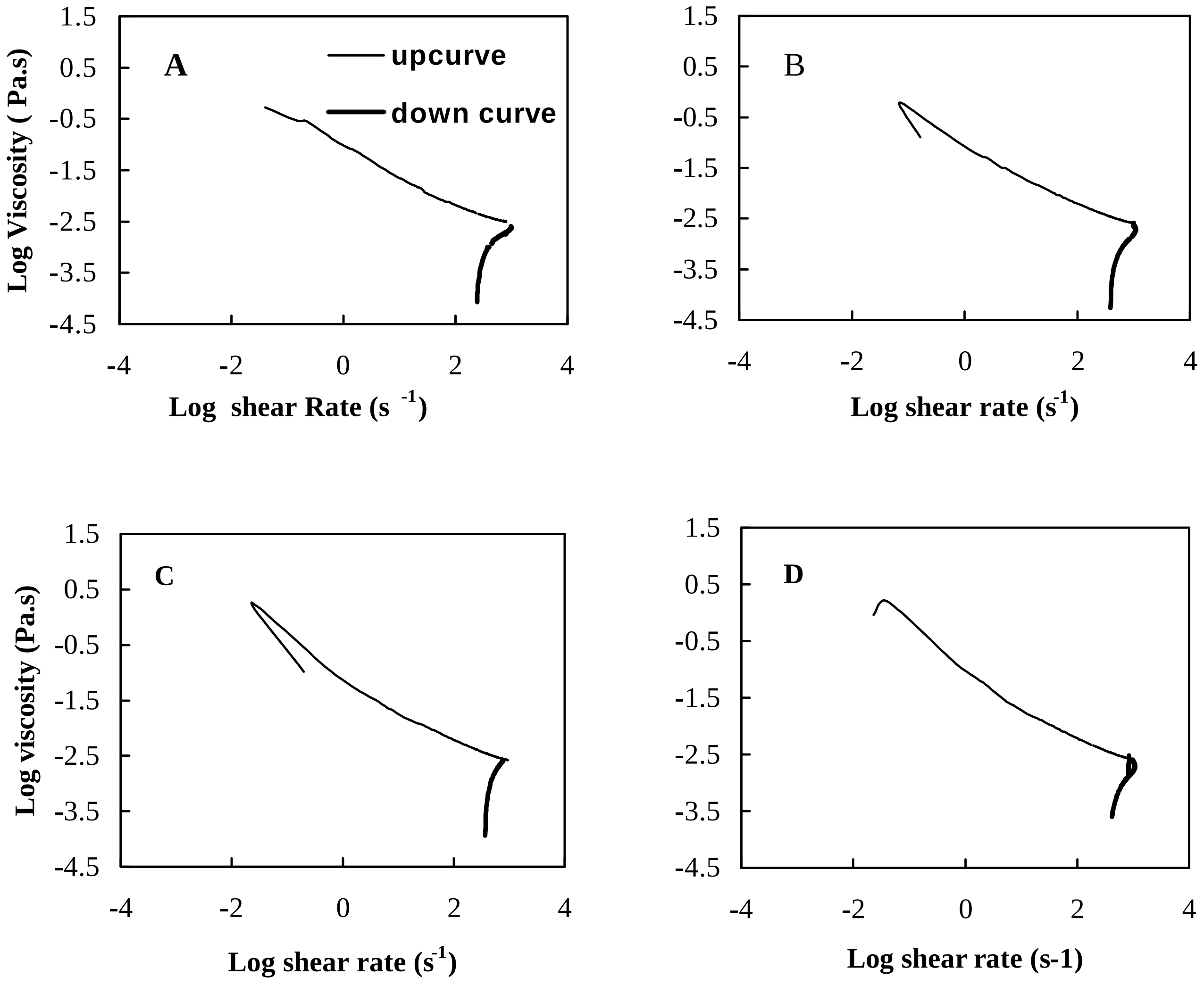

Fig. 57 : Shear stress sweep of gelatin solution (15 mg/ml) from skin of Bull's eye (*Priacanthus spp.*) A: at 25 ^{o}C B: at 40 ^{o}C C: at 50 ^{o}C D: at 60 ^{o}C

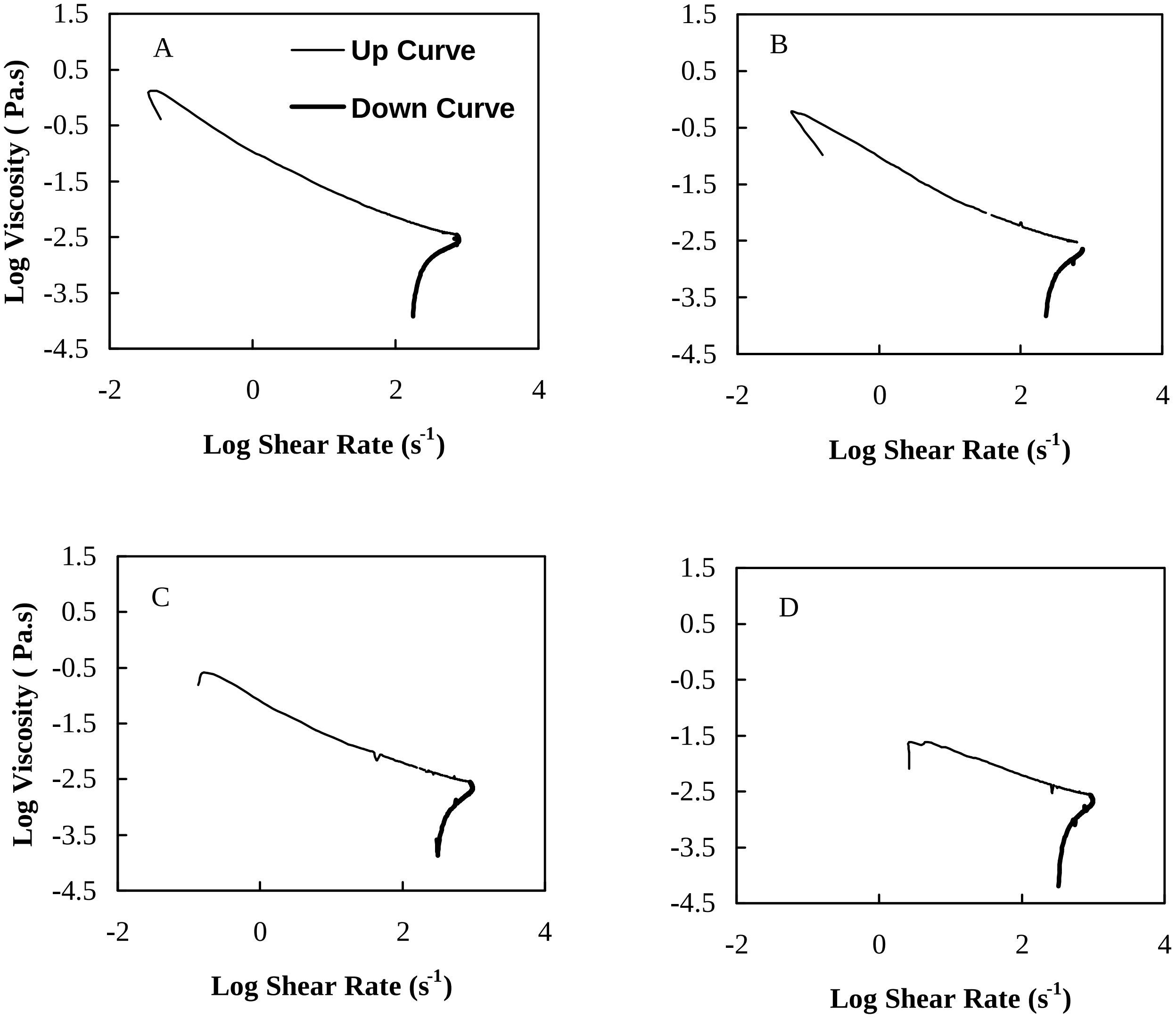

Fig. 58: Shear stress sweep of gelatin solution (30 mg / ml) skin of Bull's eye (*Priacanthus spp.*)
A: at 25 °C B: at 40 °C C: at 50 °C D: at 60 °C

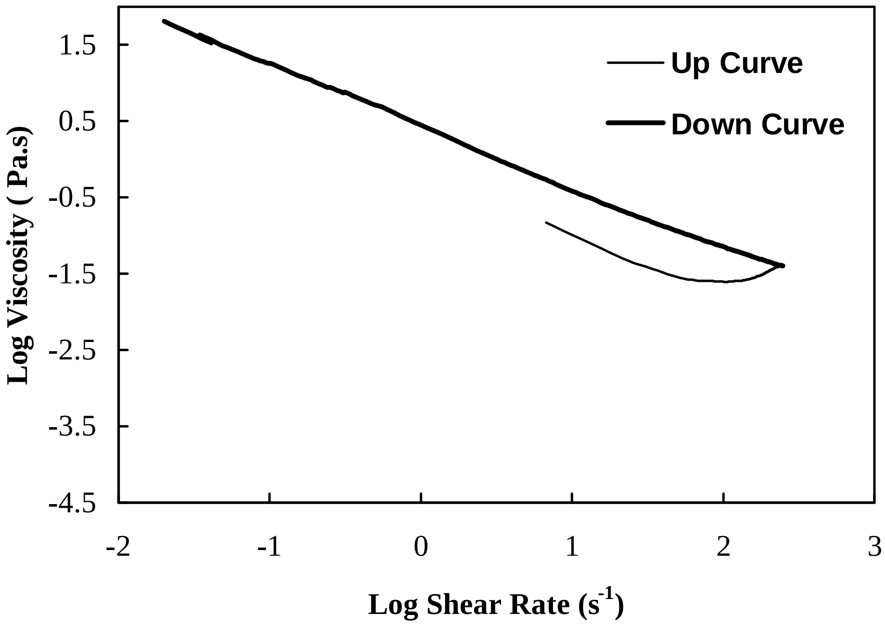

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Fig. 59: **Shear stress sweep of gelatin solution (30 mg / ml) from skin of Bull's eye (*Priacanthus spp.*) at 10 °C**

V. DISCUSSION

In the present investigation an attempt has been made to utilize low economic value fish like ribbon and bull's eye fish for the preparation of cereal based extruded products. Apart from high nutritional value, fish mince based cereal extruded products offer advantages in terms of storage and distribution at ambient temperature. Extrusion technology in recent times has become one of the major processes for producing varieties of food. Extruded foods range from breakfast cereals, snack foods with modified starches and flours to sweets. However, research on extrusion processing of fish muscle is of recent origin and is gaining importance with emphasis on utilization of by-catches and low economic value fish.

The need for utilization of fish waste especially skin, bones and viscera is gaining utmost importance so as to minimize environmental pollution. The utilization of fish skin and bones for gelatin production is attracting gelatin manufacturers as the traditional raw material source from bovine and porcine is declining due to outbreak of 'Mad cow disease'. Hence standardization of gelatin manufacture from fish skin and assessing its properties will be the means for utilization of huge fish processing waste. The main objective of the present investigation was to prepare extruded products of acceptable quality from a mixture of rice flour/wheat semolina/ragi flour and fish mince as a function of barrel temperature, screw speed and die diameter. Characterization of the raw materials used for extrusion has been carried out. Gelatin prepared from the skin of bull's eye fish (*Priacanthus spp.*) and its rheological properties were assessed.

5.1. Composition of raw materials used for extrusion

Ribbonfish (*Trichiurus spp.*) and bull's eye fish (*Priacanthus spp.*) used in the present study had fat content below 2% and they belong to lean variety fish (Table 2). The main components of fish are water, protein and lipid, which make up 98% of total weight of fish muscle. These components play a vital role in the functional property and nutritional value of the fish. The remaining 2% constitutes carbohydrate, vitamins and minerals (Ofstad *et al*., 1996). The protein content of ribbonfish and bull's eye fish was 17.91% and 16.71% respectively. The protein content of most fish averages to 15 - 20 percent (Garrow and James, 1993).

The moisture, protein, fat and ash content of rice flour used in the present study were 5.5%, 7.67%, 0.31% and 2% respectively (Table 3). The protein content of rice flour vary between 5.85 – 8.2%, while the fat content vary between 0.56 – 1.4% (Choudhury and Gautam, 1999; Ang *et al.*, 1989; Madhusudhan and Tharanathan, 1995; Spigno and Faveri, 2004). Hence, the proximate composition of rice flour used in the present study was comparable with the results of available literature. The proximate composition of wheat semolina used in the present study revealed a higher protein content (11.88%) compared to that of ragi or rice flours, while the fat content was 0.45%. Bredie *et al.* (2002) reported a protein and fat content of 9.5% and 1.5% respectively in wheat flour. The proximate composition of ragi flour showed a moisture, protein, fat and ash content of 11.37%, 8.25%, 0.28% and 4.58% respectively. The moisture and lipid content of ragi starch as reported by Madhusudhan and Tharanathan (1995) were 8.9% and 0.8% respectively. The ash content of ragi flour used in the present study was comparatively higher than that of wheat semolina or rice flour. This is due to the high content of minerals like calcium in the flour. It has been reported that calcium content of ragi is 344 mg/100g (Aykroyd *et al.*, 1966).

The aminoacid profile of ribbonfish and bull's eye fish meat revealed higher proportion of glutamic acid, alanine, lysine and leucine (Table 4). Both the fish were having higher proportion of white/light meat. The red meat of fish has more glycine, leucine, arginine and phenylalanine whereas white/light meat has more lysine, aspartic acid and glutamic acid (Garrow and James, 1993). The major amino acids present in blue whiting and codfish were glutamic acid, aspartic acid, lysine and leucine (Dagbjartsson, 1975). Fish contains significant amounts of all essential amino acids, particularly lysine. The lysine content of ribbonfish and bull's eye fish meat was 10.57 and 9.79 as percentage of amino acids. Fish protein can be used therefore to complement the amino acid pattern and improve the overall protein quality of a mixed diet.

The major fatty acid present in ribbonfish was erucic acid or cetoleic acid (C22:1n9) (16.14%) and that of bull's eye fish were heptadecanoic acid (C17:0) (12.76%) and arachidic acid (C20:0) (12.67%) (Table 5). The major fatty acid profile of these two fish was different from that of pelagic fatty fish. The major fatty acid present in sardine oil was palmitic acid (18%) followed by oleic (14.67%), Eicosa pentaenoic acid (EPA) (14.08%) and Decosa hexaenoic acid (DHA) (11.63%) (Bandarra *et al.*, 1997). The EPA and DHA content of ribbonfish and bull's eye fish were 11.83 & 7.86 and 6.99 & 8.96%

respectively, which were comparable with that of fatty fishes. Among the Poly Unsaturated Fatty Acids (PUFA) in mackerel and horse mackerel DHA was found to be predominant. This may be due to the type of food intake available to these species (Bandarra *et al*., 2001). The EPA and DHA content of Atlantic mackerel were nearly 6.23 and 14.19% respectively (Ackman and Eaton, 1971; Leu *et al*., 1981; Soriguer *et al*., 1996). Fatty fish like mackerel contains a high percentage of PUFA, where the sum of saturates will be usually less than 30% (Ackman and Eaton, 1971; Leu *et al*., 1981). In the present study the two variety of fish used were lean variety and the sum of saturates was higher (around 43%).

5.2. Differential Scanning Calorimetry (DSC)

As the extrusion process involves application of heat and pressure, it is essential to characterize the raw material like flour/starch and other ingredients like fish meat with reference to heat and pressure. Understanding the thermal properties will help in elucidating the possible interaction between different ingredients during thermal process. The DSC results are recorded as thermograms, which are plots of the differential heat input (mW/sec) against temperature. Changes in protein structure during DSC analysis are referred as 'transition' changes. The thermogram of the ribbonfish and bull's eye fish meat showed three transitions (Fig. 5A&B). For ribbonfish meat, the peak temperatures for the three transitions were at 33.17^{o}C, 48.85^{o}C and 60.96^{o}C and the corresponding transitions for bull's eye fish were at 38.35^{o}C, 47.72^{o}C and 63.02^{o}C (Table 6). The first transition was due to the denaturation of myosin, the second transition was assigned to water-soluble sarcoplasmic proteins and the third transition was due to the denaturation of actin (Badii and Howell, 2002). The DSC thermogram of arrowtooth flounder myosin showed that the onset of myosin denaturation was at 25^{o}C with two maximum transition temperatures at 30^{o}C and 36^{o}C (Visessanguan *et al.*, 2000). Thermogram of hake protein showed two transitions (T_{max} 46.5^{o}C and 75.3^{o}C) and ΔH of 4.27 cal/g (Beas *et al.*, 1990). Thermal energy absorbed by the protein will cause denaturation and give rise to endothermic effect (Tanford, 1968). The enthalpy change value for ribbonfish meat and bull's eye fish meat due to denaturation of myosin was 0.2038J/g and 0.3252J/g respectively. The enthalpy values are slightly lower than that of cod stored at –30^{o}C (Badii and Howell, 2002). DSC is a very useful means of studying the thermal properties of muscle protein and thermal denaturation (Arndfield and Murray, 1981). The behaviour of the proteins can be studied in situ and there is no need for solubilization of the sample.

The DSC profile of rice flour, wheat semolina and ragi flour showed a single transition at a temperature of 64.73^{o}C, 58.16^{o}C and 69.72^{o}C respectively (Table 6). The transition occurring in these samples are the gelatinization temperature. In the present study the DSC profile of flour/semolina samples were in solution form (10% w/v) where availability of solvent is large. Spigno and Faveri (2004) pointed that the position of the gelatinization peak was not influenced by the water content and was almost the same for all the rice starch samples with different water content. In excess solvent, co-operative swelling and solvent uptake results in complete disruption mechanism proposed by Donovan (1979) and Jenkins (1995). The gelatinization endotherm observed in DSC profile is due to granular disruption brought about by the co-operative combination of increasing solvent, thermal plasticisation and the molecular mobility (Perry and Donald, 2002). Gelatinization temperature differed between varieties, a phenomenon that only partly could be explained by variations in water content or starch granule size (Karlsson and Eliasson, 2003).

The gelatinization temperature of rice flour in the present investigation was lower than the temperature reported by Spigno and Faveri (2004). This may be due to the slow heating rate of the sample. As the heating rate increases the gelatinization temperature shifted towards higher values i.e. from 74^{o}C at 3^{o}C/min to 89.9^{o}C at 10^{o}C/min or 103.3^{o}C at 15^{o}C/min in the case of rice starch (Spigno and Faveri, 2004). With increase in heating rate the temperatures for onset, peak and conclusion also increased. It was seen that increasing the mass of the pan and decreasing heating rate would reduce the peak temperature (Yu and Christie, 2001).

The onset temperature in the DSC thermogram of wheat semolina in the present investigation was at 49.13^{o}C. Svensson and Eliasson (1995) showed that the onset temperature in the DSC thermogram of wheat starch at different moisture levels (58%, 51% and 43%) was at 44.8^{o}C. They observed the peak gelatinization temperature of wheat starch was at 57.1^{o}C. However, the gelatinization temperature of the wheat semolina used in the present investigation was slightly higher. This may be due to the higher protein content in flour compared to starch. Temperature of gelatinization for flours was found to be higher than in starches, which may be attributed to the presence of proteins, sugar and salts (Chungcharoen and Lund, 1987). Water is absorbed by these compounds, will shift the gelatinization temperature to higher side (Eliasson, 1992). Berry and White (1966) have shown that the gluten of wheat flour increases the gelatinization temperature of wheat starch.

Gelatinization is a non-equilibrium transition where granule swelling and crystallite melting (endothermic) along with hydration and crystal reorganization (exothermic) occurs. Hence the enthalpy is a net thermodynamic quantity (Biliaderis, 1990). A low temperature (≈50°C) endotherm has been reported as a common feature for polysaccharides (Gidley *et al.*, 1993). The endotherm represents the structured relaxation in a glassy amorphous polymer matrix, it could be expected that the enthalpy be positively related to the magnitude of the amorphous fraction (Chung *et al.*, 2002). The gelatinization enthalpy values of starches have been reported to be affected by factors such as granule shape and percentage of large and small granules (Stevens and Elton, 1971; Yuan *et al.*, 1993). In the present investigation wheat semolina showed highest enthalpy (0.5788 J/g) while rice flour showed the lowest value (0.2476 J/g). Lower enthalpy of gelatinization will be for starches with small granule size and lowest amylose content (Sandhu *et al.*, 2004). Kalichevsky *et al.* (1992) reported that the endothermic enthalpy of starches varied proportionally to moisture content. At a moisture content of 15%, the enthalpy value for rice starch was 0.52 J/g. Enthalpy of gelatinization was lower in flours than in starches where there is more available water for gelatinization (Eliasson, 1992; Huang *et al.*, 1994). Calzetta and Suarez (2001) and Marshell (1992) reported an increasing enthalpy of gelatinization with increasing heating rate. In the present investigation from the DSC thermogram it can be concluded that the size of starch granule is higher in wheat semolina followed by ragi and rice flour.

5.3. FT-Raman spectroscopy

Various types of structural information can be used to identify starch in the flour. In recent years vibrational spectroscopic studies of starch have been reported (Dolmatova *et al.*, 1998; Dupuy and Laureyns, 2002). Infrared and Raman spectroscopy are complementary techniques that provide information on molecular structure. In the present study FT-Raman spectroscopy has been used to characterize rice flour, wheat semolina and ragi flour both in native and gelatinized state. In addition, structural information on proteins from two selected fish species in fresh condition has been obtained. FT-Raman spectra of ribbon and bull's eye fish showed a strong band at 1005 cm^{-1} indicating phenylalanine compound. Intensity of this band is not affected by the microenvironment or external factor and can be used as an internal standard to normalize the spectrum (Tu, 1986). The α-helix content (939 cm^{-1}) and β-sheet structure (band at 990 cm^{-1} and 1239 cm^{-1}) in ribbonfish and bull's eye fish meat showed almost similar intensity (Table 7&8). FT-Raman spectra has been used to understand protein-protein interaction wherein decrease in α-helix and increase in β-sheet structure are reported (Howell and Li-Chan, 1996). The sharp peak at 760 cm^{-1} in both ribbonfish and bull's eye fish is due to indole ring of tryptophan.

In FT-Raman spectra several bands are assigned to tyrosine residues, which play an important role in hydrogen bond formation in the protein molecules. Among these residues the tyrosine doublet band, which is located at 830 and 855 cm^{-1} are very useful for monitoring the protein conformation (Li-Chan *et al*., 1994). The alteration in this region is due to disruption of hydrogen bonds formed by tyrosine residues which interact with the surrounding water environment and act as a hydrogen acceptor or donor (Parker, 1983; Li-Chan *et al.*, 1994; Ogawa *et al*., 1999). The ratio of the bands I_{855}/I_{830} is high (0.9-1.45) if the tyrosine residue is exposed, and low in the presence of strong hydrogen bonding. The ratio of the bands I_{855}/I_{830} for ribbonfish was 0.64 and that of bulls eye fish was 0.80, indicating that tyrosine residues are not exposed.

The secondary structure of protein can be revealed by FT-Raman spectra. The band assigned at wave number of 1660 cm^{-1} is Amide I, which indicates undefined or random coil structure. Both ribbonfish and bull's eye fish had Amide I at a wave number of 1660 cm^{-1} indicating the presence of undefined or random coil structure. Presence of α-helix and β-sheet structure is obtained if the Amide I band is centered at 1645-1657 cm^{-1} and 1665-1680 cm^{-1} respectively (Li-Chan *et al.*, 1994). In the present study the proportion of α-helix and β-pleated sheet of ribbonfish and bull's eye fish was found to be less than what one would have expected (Table 7&8), probably because of use of frozen and thawed fish for the study. The use of frozen fish for Raman spectra study was necessitated as the facility available for Raman spectra analysis was at a distant place.

The FT-Raman spectra of native rice flour/wheat semolina and ragi flour revealed a conspicuous peak at a wave number of 480 cm^{-1} and hence the intensity of this wave number is used as internal standard for normalization of the spectra. The appearance of an intense band at a wave number of 480 cm^{-1} is a characteristic feature due to α-(1-4) bonds of polysaccharides (Zhbankov *et al.*, 2004). The C-H stretch and CH_2 deformation of the corn starch has been attributed to amylopectin content (Zhbankov *et al.*, 1997). In the present study the intensity of CH_2 deformation in rice flour, wheat semolina and ragi flour were 0.47, 1.6 and 0.57 respectively. The results clearly indicate a higher amylopectin content in wheat semolina. Further, wheat semolina samples showed two additional peaks between wave number 1663-1630 cm^{-1} and another between 1595-1528 cm^{-1} corresponding to Amide I and Amide II respectively (Fig. 12). This is mainly due to the gluten, a protein in wheat semolina present in higher proportion as revealed by proximate composition studies (Table 3). Thygesen *et al.* (2003) reported similar results while analyzing wheat flour by FT-Raman spectroscopy. The peak arising due to Amide I in rice and ragi flour was rather small due to less protein content.

In FT-Raman spectra of the flour/semolina after gelatinization was carried out and the same is represented in the Fig. 11, 13 & 15. The gelatinization was carried out by heating the flour/semolina suspension at 80^oC for 30 minutes. The gelatinized flour (paste) was used for FT-Raman spectra study. The intensity of bands in the gelatinized wheat semolina and ragi flour decreased considerably in comparison to ungelatinized flour, however the intensity of band at wave numbers of 1633 cm^{-1} and 3213 cm^{-1} in rice flour, wheat semolina and ragi flour has increased in gelatinized flour. This can be attributed to the uptake of water into the crystalline structure of the starch, and following, the gel formation, which causes the break of intramolecular and intermolecular hydrogen bonds due to the uptake of water into the starch particles and the formation of new hydrogen bonds with water (3213 cm^{-1}) (Schuster *et al.*, 2000).

The Raman spectrum of gelatinized rice flour was different from that of gelatinized wheat semolina and ragi flour. The intensity of bands in the spectra of gelatinized rice flour was almost same as that of ungelatinized rice flour except that the intensity of bands appearing at 1633 cm^{-1} and 3213 cm^{-1} where it increased. It is not clear as to why the intensity of bands remained almost constant even after gelatinization. From the FT-Raman spectra results it is evident that gelatinization of starch from 3 different sources has decreased the intensity of several bands and also by increasing water signals. Since the samples in the present study were used in the form of flour or grits (wheat semolina) the spectra looks complicated due to involvement of other constituents in the sample. An attempt has been made to compare the intensity of bands what is available in the literature has been mainly focused on starch constituent. The vibrational spectra are especially sensitive to the geometry of the molecules, system of intra molecular and inter molecular interactions. This has determined the significance of vibrational spectroscopy in investigating structure and properties of carbohydrates.

5.4. Small deformation testing of flours/semolina solutions

Understanding the process of gelatinization can be complimented by dynamic rheological testing in addition to spectroscopy and microscopy. Dynamic rheological properties of flour samples (10% w/v solution) revealed that the maximum G' (storage modulus) for rice flour, wheat semolina and ragi flour were 6029 Pa, 118 Pa and 544 Pa respectively (Fig. 16A,B&17). The difference in G' and G'' during the heating may be attributed to the difference in the starch granule structure which in turn depends on their biological origin (Svegmark and Hermansson, 1993). The rheological properties of starch depended mainly on the interaction among closely packed granules and their rigidity during the heating process (Sandhu *et al.*, 2004). Rheological properties of starches also depend on starch concentrations. In a concentrated regime, starch granules cannot swell to their equilibrium volume, as the water availability is limited. The rheological features of starch suspensions are then mainly determined by the particle rigidity of the swollen granules (Vandeputte *et al.*, 2003). It is necessary to point out that in starch gels, a three-dimensional network is built from amylose macromolecules, whereas, amylopectin plays the role of the filler (Yuryev *et al.*, 1995). For all the flour samples in the present study G' values increased with increase in temperature. The initial increase of G' could be attributed to the degree of granular swelling to fill the entire available volume of the system (Eliasson, 1986; Keetles and Van Vliet, 1994) and intergranule contact might form a network of swollen granules (Evans and Haisman, 1979; Wong and Lelievre, 1981). The sharp increase in G' values in all the samples was between 60^{o}C to 70^{o}C. The lowering of G' with further increase in temperature could be attributed to the melting of remaining crystallites, which resulted in swollen granules to become softer (Tsai *et al.*, 1997). Hence, continuous heating provided the energy to breakdown the residual crystalline structure of the granule. The extent of decrease in G' is a degree of disintegration of starch granules (Singh *et al.*, 2002).

The temperature at which the G' and G'' cross over is referred as cross over temperature which is nothing but gelatinization temperature. The temperature at which cross over has taken place for rice flour, wheat semolina and ragi flour during dynamic rheological testing was found to be at 61.63°C, 60.22°C and 52.68°C respectively (Table 11). This cross over temperature is comparable to the peak temperature in the DSC thermogram. From the results of rheology and DSC thermogram it is evident that gelatinization temperature of rice flour, wheat semolina and ragi flour can be taken as 64.7°C, 58.16°C and 69.72°C respectively.

Upon cooling during dynamic rheological testing the G' values of rice flour increased up to 40°C and further cooling, the values decreased. Similarly for ragi flour increase in G' was recorded up to 30°C and further reduction in temperature the G' value decreased. Wheat semolina showed gradual decrease in G' during cooling from 90°-20°C. The decrease in G' value during cooling regime has been attributed to concentration of gel volume at certain critical temperature (Tsai *et al.*, 1997). However, Yang *et al.* (2004) observed that as the temperature decreased, G' for pure wheat starch gel increased. Ring (1985) showed that starch and water dispersions heated above their gelatinization temperature behaved as viscoelastic pastes and upon cooling the paste thickens and may form an elastic gel if the dispersion has sufficient concentration.

5.5. Phase contrast microscopy of flours/semolina

Phase contrast microscopy photographs of rice flour, wheat semolina and ragi flour were obtained in powdered form, wet state (ungelatinized) and gelatinized state. The shape of starch granules was found to be irregular varying from round to polyhedral. All the granules were found to be intact and there was no disruption in shape (Fig. 18-20). Various researchers have reported that shape and size of starch granules from rice and ragi using scanning electron microscopy technique (Hoover *et al.*, 1996; Madhusudhan and Tharanathan, 1995). For rice flour the shape has been found to round, angular and polyhedral with average dimension of 2-8 μm. For ragi flour the granule size varied from 1-9 μm and the shape range from spherical to hexagonal / polygonal. The difference in granule morphology (round, oval and polyhedral) may be attributed to the biological origin, biochemistry of the amyloplast and physiology of the plant (Svegmark and Hermansson, 1993). In the present investigation phase contrast microscopy revealed that the granule size of wheat semolina was higher than rice or ragi flour (Fig. 19). Olkku and Rha (1978) reported that the size of wheat starch granules can vary from 2-35 μm.

The phase contrast microscopic photograph of the flours in wet state showed swelling of the granules (Fig. 18B, 19B & 20B). Prior to gelatinization, water will be imbibed by the granules and the process has been reported to be reversible (Kerr, 1950). The structure of the starch granules will be unaltered in presence of water prior to onset of gelatinization (Gough and Pybus, 1971).

The photographs of gelatinized rice flour, wheat semolina and ragi flour is given in Fig. 18C, 19C & 20C. The granule swelling for wheat semolina was higher as compared to rice and ragi flour. The swelling power of starch has been reported to depend on water holding capacity of starch molecules by hydrogen bonding (Lee and Osman, 1991). Hydrogen bonds stabilizing the structure of the double helixes in crystallites are broken during gelatinization and are replaced by the hydrogen bonds with water and swelling is regulated by the crystallinity of the starch (Tester and Karkalas, 1996). Starch granules with low amylose content being less rigid swell freely when heated (Sandhya Rani and Bhattacharya, 1989). The major factor in starch gelatinization is granule swelling which depends on the strength and character of the micellar network within the granule, which in turn is dependent on the degree and kind of association (Govindaswamy *et al*., 1996). These swollen granules are mainly composed of amylopectin, where the continuous phase consists mainly of amylose (Sasaki *et al*., 2004). With the continued swelling of granules, starch particles that have become fully hydrated separate themselves from the intricate micellar network and diffuse into the aqueous medium (Olkku and Rha, 1978). Protein and starch form complexes in the flour while being gelatinized. According to Takeuchi (1969) the starch protein interaction is due to the attraction of opposite charges in the system.

The swelling of starch granules will lead to attainment of viscosity, which in turn is responsible for binding with other constituents in the food system. The process of gelatinization assumes greater significance in the process of extrusion as well as mince-based products. The present investigation is aimed at extrusion of cereal based fish mince products; it is of importance to understand protein-polysaccharide interaction during heating. The results of dynamic rheological testing of fish protein-polysaccharide mixture have been discussed in the next section.

5.6. Protein-polysaccharide interaction

The protein-polysaccharide interaction studies were carried out with proteins extracted from ribbonfish meat, using extraction buffer as detailed in Material and Methods section 3.2.8, and flour/semolina at different temperatures using control stress rheometer. A time sweep was performed at 3 different temperature viz. 60°C, 70°C and 80°C and parameters like G’, G’’ and tan δ were recorded.

Time sweep of rice flour solution (20% w/v) revealed that G’ values decreased with time at 60°C and 80°C (Fig. 21). However, at 70°C storage modulus (G’) increased with time period and the maximum G’ (102.6 KPa) was observed. Tan δ values decreased in all the cases. With the addition of protein solution from ribbonfish meat G’ values decreased. Protein in the solution will compete for the available water during the process of gelatinization of starch. This leads to incomplete gelatinization of starch as available moisture is reduced, with the result the G’ values of mixture of protein and flour as a function of time is decreased. Tan δ values also showed an increasing trend indicating more of viscous element than that of elastic network. Protein-polysaccharide interactions are not specific classes of interactions but are typical of the types of interactions that may occur between different types of polymer in solution (Ledward, 1994).

G’ value increased with time period in the case of wheat semolina solution (20% w/v) irrespective of the temperature (Fig. 22). The maximum G’ value (8.25 KPa) was observed at a temperature of 70°C. Tan δ values showed a decreasing trend in all cases indicating formation of gel structure. Addition of protein solution from ribbonfish meat enhanced the G’ value irrespective of the temperature. With the addition of protein solution, the maximum G’ value observed at 70°C was 16.05 KPa. An increase in G’ of values of protein-starch mixture indicates that starch has synergistic effect in enhancing the gel network. It also should be noted the wheat flour had a higher protein content and possibly involvement of gluten in enhancing the gel network cannot be ruled out. Higher G’ values obtained for flour-protein mixture at 70°C arises due to opening up of the myofibrillar proteins from fish and facilitating ordered aggregation. Further low phase angle or tan delta clearly indicates that the elastic modulus predominates over the viscous modulus, meaning strong gel (Sasaki *et al.*, 2004).

Time sweep of ragi flour solution (20% w/v) at 70^{o}C and 80^{o}C showed an increase in G' value with increase in time period. However, at 60^{o}C the G' values showed a decreasing trend with time (Fig. 23). The maximum value for G' (81.71 KPa) was observed at 80^{o}C. The mixture of ragi flour-protein from ribbonfish meat showed an increase in G' values during time sweep experiment. The maximum G' value (134.9 KPa) was observed at 70^{o}C. During the time sweep the tan δ values progressively decreased indicating elastic network. As in the case of wheat semolina, the maximum G' value for ragi flour-protein mixture was obtained at 70^{o}C which clearly indicates the gel forming ability of ribbonfish meat could be enhanced in presence of ragi flour possibly by binding of proteins to viscous starch. Ribbonfish meat in presence of wheat semolina/ragi flour has a positive interaction in terms of development of elastic modulus, which may have a bearing during extrusion process. When different polymers are mixed, chemically dissimilar polymer tends to be incompatible for thermodynamic reasons. Phase separation between amylose and added hydrocolloids occurs in the continuous phase due to thermodynamic incompatibility (Alloncle and Doublier, 1991; Kulicke *et al.*, 1996). Phase separation resulting from incompatibility between unlike polymers influences the firmness of the mixture gel (Sasaki *et al.*, 2004). Yoshimura *et al.*, (1996) indicated that adding hydrocolloids increased the effective starch concentration by immobilizing water molecules, leading to a more stable structure of the mixture gel. Mixing of dissimilar macromolecules causes phase separation and influences the water partition between the two phases. Effective concentration after phase separation is higher than the initial concentration of the two polymer constituents (Kasapis, 1995). In the present study the dynamic rheological testing of fish protein-ragi flour/wheat semolina mixture clearly indicated compatibility of the two polymers as revealed by low tan δ values and high G' values.

Analyzing the dynamic rheological testing of flour/semolina alone, there exists a difference in rheological parameters like G' and G''. The variation in G' values for rice flour, wheat semolina and ragi flour may be due to the differential composition of amylose content. Amylose restrains starch granule swelling and helps reduce loss in granular rigidity (Tsai *et al.*, 1997). The rigidity of swollen starch granules is a major factor in determining the formation of gel, which suggests that a slight difference in amylose content contributes to the elastic component of starch gels (Parovuori *et al.*, 1997; Sasaki *et al.*, 2002). Higher the amylose content, lower the tan delta and favoring gel network (Sasaki *et al*., 2004).

5.7. Extruded products

5.7.1. Proximate composition of extruded products

In the present investigation extruded products were prepared using ribbonfish and bull's eye fish meat in combination with rice flour, wheat semolina and ragi flour. The process variables such as barrel temperature, screw speed and die diameter and concentration of fish meat were studied so as to get acceptable products. This section deals with composition and quality of extruded products as a function of extrusion process variables. The reasons and mechanism for changes in quality and composition have been discussed.

Function of barrel temperature

Extruded products were prepared at a constant screw speed of 350 RPM using a die diameter of 4 mm as a function of barrel temperature from the flour/semolina-fish mince mixture. The proximate analysis of extruded products from rice flour, wheat semolina and ragi flour revealed that with an increase in barrel temperature there was a reduction in moisture content irrespective of the flours used (Table 12). Higher extrusion temperatures resulted in lower moisture content in the extruded products from triticale (Lorenz *et al*., 1974). Moisture content of extruded products with fish mince showed a higher value than control samples. At any given barrel temperature the moisture content of extruded products with 20% fish mince showed a higher value. This is because of the higher moisture content in the raw material itself.

The protein content of extruded rice flour-fish mince (10% level) mixture varied between 7.84-8.47%. With 20% fish mince the protein content varied between 9.57-10.5%. Protein content of the control sample ranged between 6.51-6.73%. Hence, the higher protein content in the extruded product with fish mince is attributed from the protein content of fish mince. Fat content of all the samples were less than 1%. The increase in fat content with increase in barrel temperature may be due to the reduction in moisture content of the product. The higher protein content in the wheat semolina based extruded products revealed the higher gluten content in wheat. The protein content in the control wheat semolina sample itself varied between 10.10-10.55%. Extruded ragi flour based products showed a higher percentage of ash content. It is well documented that ragi flour is rich in calcium and soluble fibre (Gopalan *et al*., 1969). Hence higher ash content was found in the ragi flour based extruded products.

Function of screw speed

Extruded products were prepared at a constant barrel temperature of 90^{o}C using a die diameter of 4 mm as a function of screw speed. The moisture content of extruded products from rice flour, wheat semolina and ragi flour with 10% ribbonfish mince increased with an increase in screw speed (Table 13). With an increase in screw speed there will be reduction in residence time, which will reflect in moisture content. The short residence time may be the reason for higher moisture content in the products prepared at higher screw speed. The marginal reduction in fat content with increase in screw speed is due to the increase in moisture content. The difference in protein content of extruded products as a function of screw speed was marginal.

Function of die diameter

Extruded products were prepared at a constant barrel temperature of 90^{o}C and screw speed of 350 RPM as a function of die diameter. Proximate composition of extruded products from a mixture of rice flour/wheat semolina/ragi flour and 10% ribbonfish mince prepared as a function of die diameter revealed that the moisture content increased both in the control and samples with an increase in die diameter (Table 14). With an increase in die diameter the pressure developed inside may get reduced. The reduction in pressure in the die end may be the reason for higher moisture content of extruded products prepared using a larger die diameter. In the extrusion processing of triticale, Lorenz *et al.* (1974) observed an increase in moisture content of the extruded products with an increase in die diameter.

It should be noted that the proximate composition of extruded products from the mixture of ribbonfish and rice flour, wheat semolina and ragi flour as a function of screw speed and die diameter was carried out with ribbonfish mince only (10%).

5.7.2. Aminoacid composition of extruded rice products with added fish mince

Aminoacid composition of extruded products from rice flour – fish mince mixture were analyzed. The fish used included both ribbon and bull's eye fish at 10% and 20% level. The process parameters included a constant barrel temperature of 90^{o}C and screw speed of 350 RPM using a die diameter of 4 mm. Aminoacid composition of extruded products from a mixture of rice flour-fish mince (ribbonfish and bull's eye fish mince) mixture showed a higher proportion of glutamic acid, alanine, tryptophan, leucine and lysine (Table 15). The higher proportion of glutamic acid, alanine, leucine and lysine in the extruded products accounted for the aminoacid content of the added fish mince. Fish contains significant amounts of all essential amino acids, particularly lysine in which cereals are relatively poor (Garrow and James, 1993). With increase in percentage of fish mince from 10% to 20%, the percentage of essential aminoacids especially lysine increased. Lysine content was higher in extruded product prepared with 20% ribbonfish mince.

5.7.3. Expansion ratio of extruded products

Function of barrel temperature

Extruded products were prepared at a constant screw speed of 350 RPM using a die diameter of 4 mm as a function of barrel temperature. The expansion ratio of rice flour based extruded products increased with an increase in barrel temperature irrespective of the percentage of fish mince added (Table 16 A, Fig. 30), however, the increase was not significant ($P<0.05$) (Table 16 B). Maximum expansion ratio (375 %) was observed in product with 10% ribbonfish mince prepared at a barrel temperature of 120^{o}C, while that of control had an expansion ratio of 299%. The incorporation of bull's eye fish mince to rice flour for extrusion process, the expansion ratio followed similar trend to that of the products with ribbonfish mince, however the extent of expansion ratio was much lower.

The extrusion process with incorporation of 20% fish mince at 120^{o}C could not be carried out as the mixture used to get stuck inside the barrel without extruding it out. Hence incorporation of fish mince at 10% level was carried out at 3 different barrel temperature *viz.* 70^{o}C, 90^{o}C and 120^{o}C. Incorporation of fish mince at 20% for extrusion process was carried out at a barrel temperature of 70^{o}C and 90^{o}C only.

The expansion ratio of extruded products from a mixture of rice flour-fish mince at 20% (ribbonfish/bull's eye fish) decreased compared to products obtained from 10% fish mince and control samples. This is mainly due to the slightly higher moisture content and improper gelatinization, which may hinder expansion process. It should be noted that the composition of fish and rice flour mixture prior to extrusion reveals a difference of 1-1.5% moisture level when higher percentage of fish mince was added. According to Yacu (1995) overall expansion is a function of die temperature. Low die temperature produces less flashing, resulting in poor overall expansion. The low expansion ratio of the extruded products prepared at a lower barrel temperature in the present investigation may be due to lower degree of gelatinization. Extrudate expansion is influenced by degree of gelatinization (Mercier and Feillet, 1975; Chinnaswamy and Hanna, 1990: Chinnaswamy and Bhattacharya, 1983, 1984; Cai and Diosady, 1993), starch breakdown (Gomez and Aguilera, 1983, 1984; Kirby *et al.*, 1988; Cai and Diosady, 1993) and amylose content (Chinnaswamy and Hanna, 1990). The expansion process can be described as nucleation in the die, extrudate swelling immediately beyond the die, followed by bubble growth and collapse (Kokini *et al.*, 1991). The expansion of extrudates can be due to two phenomena: an elastic recovery of the melt flow and expansion of the extrudates as a result of vapour release. The former is affected by the normal stresses occurring when the melt is flowing and its relaxation when melt flow conditions change at the die outlet. This phenomenon is known as a Barrus effect. Expansion of the extrudates due to vapor release also occurred at the die outlet and it can be explained through a sharp change in the external pressure acting on the melt (Yuryev *et al.*, 1995).

The expansion ratio of extruded products from wheat semolina and ribbonfish mince at 10% and 20% revealed that the values were lower than that of control. With reference to expansion ratio it appears use of wheat semolina-ribbonfish mince mixture may not be compatible for extrusion process. One of the possible reasons is antagonistic reaction of proteins from wheat semolina and ribbonfish mince impairing the gelatinization process and reducing the expansion ratio. The rheological data on wheat semolina-ribbonfish protein interaction indicated higher G' values at 70^{o}C. Theoretically, one would have expected complete gelatinization during extrusion process leading to a desired quality characteristic like expansion ratio. Since the extrusion process of wheat semolina-ribbonfish mince has not yielded higher expansion ratio, it appears the mechanical shear during extrusion might have had the influence in reducing the expansion ratio. The expansion ratio of products from wheat semolina increased significantly ($P<0.05$) with the addition of 10% bull's eye fish mince (Table 16 B).

The expansion ratio of the products obtained from a mixture of ragi flour and fish mince followed the same trend of wheat semolina-fish mince mixture. Choudhury and Gautam (2003) observed a reduction in radial expansion from 11.71 to 9.4 with the addition of fish solids at 5% level. They also observed with further addition of fish solid (10% & 15%) there was only slight reduction in radial expansion. Increasing the amount of pink salmon solids in blends with rice flour reduced the radial expansion considerably (Gogoi *et al*., 1996a, 1996b; Gautam *et al*., 1997; Choudhury *et al*., 1998). Clayton and Miscourides (1992) reported a reduction in radial expansion upon addition of fish solids (Atlantic cod) to rice flour. Suppression of product expansion was observed when the amount of soy protein and wheat gluten was increased during extrusion of corn starch (Mohamed, 1990) and wheat starch (Faubion and Hoseney, 1982) respectively. Expanded volume is related to a combination of extrusion processing variables and raw material qualities (Chinaswamy and Hanna, 1990).

Function of screw speed

The expansion ratio of the extruded products as a function of screw speed has been assessed for flours/semolina-ribbonfish mince (10%) and flour/semolina without fish mince (Table 17 A). Variation of screw speed was coupled with constant barrel temperature of 90^{o}C and a die diameter of 4mm. This was necessitated as the expansion ratio of the products as a function of barrel temperature revealed at 90^{o}C and ribbonfish at a concentration of 10% gave a higher expansion ratio. Hence keeping barrel temperature at 90^{o}C and die diameter of 4 mm, screw speed was varied from 300-500 RPM. Further only ribbonfish mince was used in combination with flour/semolina. Expansion ratio of extruded products from rice flour, wheat semolina and ragi flour showed a decreasing trend with increase in screw speed (Fig. 31). Addition of fish mince further caused reduction in expansion ratio irrespective of flours used, however, the variation was not significant at 5% level of significance (Table 17B). Increase in screw speed decreased the retention time of the sample in the extruder which cause decreased starch gelatinization (Chiang and Johnson, 1977; Della Valle *et al.,* 1987). The decreased retention time of the sample in the extruder reduced the extent of cooking and presumably resulted in the decrease of starch gelatinization. Ilo *et al*. (1999) stated that screw speed influenced the expansion negatively in the radial direction. This can be explained by the positive effect of screw speed on shear stress. Increased shear stress with increasing screw speed in extrusion cooking induces the breakdown of the starch molecules that decrease the elasticity of the melted material. This decreases, the extrudate expansion in the sectional direction (Ilo *et al*, 1999). In the extrusion of sago starch, increasing screw speed increased shear rate but also lowered the residence time, which reduces swelling making the granule less susceptible to shearing action (Govindaswamy *et al*., 1996).

Function of die diameter

The variation in die diameter in the present investigation ranged from 3-5 mm. While varying the die diameter the barrel temperature was kept at 90^{o}C with a screw speed of 350 RPM. This was done taking into consideration the results from variation in screw speed. The range of extruded products included different flour/semolina-ribbonfish (10%) mixture and flour/semolina without fish mince. Expansion ratio of extruded products from rice flour, wheat semolina and ragi flour with 10% ribbonfish mince as a function of die diameter revealed that with an increase in die diameter there was a significant ($P<0.05$) decrease in product expansion ratio in the case of rice and ragi flour based extruded products (Table 18 B). The maximum expansion (425 %) among the products with fish mince was shown by rice flour extruded using a die diameter of 3 mm. With increase in die diameter the pressure generated near the die end will get reduced. This reduction in pressure at the die end may be reason for lesser expansion of the product.

5.7.4. Water absorption capacity of extruded products

Function of barrel temperature

Water absorption capacity (WAC) of extruded products from rice flour increased with an increase in barrel temperature (Table 19 A). The maximum value of 6.99 g water/g dry material was for the sample with 10% ribbonfish mince prepared at a barrel temperature of 120^{o}C. Water absorption index (WAI) progressively increased with an increase in extrusion temperature during the extrusion processing of long grain rice flour and tripled as the extrusion temperature increased from 70^{o}C to 120^{o}C (Kadan *et al*., 2003). In the case of short grain rice flour, WAI stayed unchanged up to 100^{o}C and at higher extrusion temperature it often decreased. Reports showed that an increase in moisture content during extrusion had little effect on WAI (Abdel-Aal *et al*., 1992; Bryant *et al.*, 2001). In the extrusion cooking of sorghum, WAI increased progressively with barrel temperature up to about 188^{o}C to 193^{o}C and then decreased as decomposition or degradation began to take place (Anderson *et al.*, 1969). Water absorption capacity of extruded samples in the present investigation decreased when fish content was increased to 20%. In the extrusion process of corn, rice and potato flour with addition of whey protein concentrate revealed that increasing whey product concentration beyond 25%, water absorption increases significantly (Onwulata *et al.*, 1998). In the present study the water absorption capacity of extruded products obtained from 10% fish (ribbonfish/bull's eye fish) with flour/semolina gave a higher values indicating involvement of protein in enhancing WAC, however with increase in fish mince concentration up to 20% WAC values were not higher when the barrel temperature was 70^{o}C. Lower water absorption capacity of extruded products from rice flour and 20% fish mince is possibly due to incomplete gelatinization because of higher concentration of protein.

In case of wheat semolina based extruded products control samples had higher water absorption capacity values. Addition of 10% bull's eye fish mince caused significant ($P<0.05$) reduction in water absorption capacity (Table 19 B). Maximum water absorption capacity (7.04 g water/g dry matter) was recorded for the wheat control prepared at a barrel temperature of 120^{o}C. In the extrusion cooking of wheat semolina, when the temperature was increased to 125^{o}C, the amount of damaged starch increased very sharply, thereby decreasing the water absorption capacity of the products (Kim and Rottier, 1980). Addition of fish mince at 20% (ribbonfish/bull's eye fish) decreased the water absorption capacity values significantly at a temperature of 70^{o}C and 90^{o}C. It is likely that damage to starch molecule at these two temperatures will be minimum and reduction in WAC values in all probability is due to incomplete gelatinization in the mixture. Further availability of moisture is a critical parameter for complete gelatinization and in the present study the maximum of 16% moisture was made available which might have impaired the complete gelatinization process. Hence reduction in WAC values has been observed.

The water absorption capacity of ragi based extruded products were comparatively lower than that of rice flour or wheat semolina based products. The maximum value was shown by the product with 10% bull's eye fish mince prepared at a barrel temperature of 120^{o}C. Addition of ribbonfish mince at 20% caused considerable reduction in the WAC of ragi based extruded products (Fig. 33).

Function of screw speed

Water absorption capacity (WAC) of rice flour/wheat semolina/ragi flour with 10% ribbonfish mince prepared at a constant barrel temperature of 90^{o}C using a die diameter of 4 mm as a function of screw speed has been evaluated (Fig. 34). The WAC of control samples (without fish mince) increased with increase in screw speed. Addition of 10% ribbonfish mince to rice and ragi flour significantly reduced the water absorption capacity as a function of screw speed. The WAC values of extruded wheat semolina-fish mince mixture showed significantly ($P<0.05$) higher values with increase in screw speed over that of control (Table 20 B). Govindaswamy *et al*., (1996) reported that the water absorption index (WAI) values of sago starch increased with increase in screw speed and opined that elevation of shear rate with increase in screw speed resulted in structural modification leading to higher WAI values. In the present study the higher water absorption capacity values in wheat semolina-fish mince extruded products is possibly due to modification of gluten and starch at higher screw speed.

Function of die diameter

Water absorption capacity of extruded products prepared from flour/semolina at a constant barrel temperature of 90^{o}C and screw speed of 350 RPM as a function of die diameter has been analysed. The die diameter had a great influence in reducing the water absorption capacity of extruded wheat semolina control and wheat semolina-ribbonfish mince (10%) mixture. At the same time the die diameter did influence in increasing the WAC values of rice and ragi both in control and ribbonfish mince based samples (Fig. 35). However, this increase in WAC values as a function of die diameter was not significant ($P<0.05$) (Table 21 B). It is generally believed that degradation of starch and denaturation of protein can cause significant reduction in WAC values (Gomez and Aguilera, 1983; Shamasundar and Prakash, 1994). Lower die diameter probably would have increased the shear inside the barrel and would have altered starch molecules and protein leading to lesser WAC in the extrudates.

5.7.5. Color of extruded products

Color of the extruded products is one of the important attributes in determining the final quality of extruded products. Instrumental analysis of color of the food products has been practiced for many processed foods and they are adjunct as an objective method. In the present study the color of extruded products obtained from flours/semolina-fish mince mixture and the respective control samples have been assessed using Hunter's colorimeter.

As a function of barrel temperature

In the present study extruded products from rice flour-bull's eye fish (20%) at a barrel temperature of 90^{o}C gave a L* value of 70.76 which was higher than the products containing 10% and 20% ribbonfish mince and 10% bull's eye fish mince (Table 22A). It should be noted that extrudates obtained from rice flour alone at a barrel temperature of 90^{o}C gave a L* value of 73.26 and decreased to 55.22 when the barrel temperature was 120^{o}C. The value of L* is the most important of the three-color coordinates because it indicates the lightness/darkness of the sample, a property that correlates best with the degree of processing (Apruzzese *et al*., 2000). Ames *et al.* (1998) have analyzed Maillard reaction products in an extrusion cooked model system using wheat starch Type A. They have indicated adding L-lysine monohydrochloride along with starch during extrusion process might be responsible for Maillard reaction in the products, which affects the color of the extrudates. Apruzzese *et al*. (2000) reported that with an increase in barrel temperature at a constant screw speed leads to darker products in the extrusion of corn flour. It is difficult to pin point the reason of higher L* value with addition of fish mince at higher temperature and the most likely reason is interaction of starch and constituents of fish muscle.

Addition of 10% ribbonfish to the rice flour increased the a* value with increase in barrel temperature. However, addition of 20% ribbonfish to the rice flour with a barrel temperature of 90^{o}C decreased the a* values significantly with increase in barrel temperature. At 20% level of bull's eye fish a higher a* value was obtained at a temperature of 70^{o}C. Incorporation of ribbonfish and bull's eye fish mince to the rice flour did not alter significantly the b* values as a function of barrel temperature. The type of fish and the concentration used for extrusion process apparently increased the b* values over that of control. But the values were not found to be significant ($P<0.05$). The extruded products obtained from dehulled oats using a single screw extruder exhibited a higher a* and b* values at higher temperature (Gutkowski and El-dash, 1999). The higher a* and b* values are indication of darkening in the product presumably due to formation of Maillard compounds. Ames (1992) proposed that Maillard reaction is primarily responsible for color development when most foods are heated. It is also postulated that low moisture content and high temperature conditions during extrusion favour Maillard reaction leading to darkening of the extrudates (Lea and Hannan, 1949). Color of extruded triticale became darker with increase in extrusion temperature (Lorenz *et al*., 1974). Though many reactions take place during extrusion cooking affecting color, the most widely accepted hypothesis is non-enzymatic browning reaction and pigment degradation. In the present investigation based on a* and b* values the extrudates obtained from rice flour-fish mince mixture can be treated as dark products. However, the overall appearance still gave a light color, possibly L* values overriding a* and b* values. The extrudates obtained from rice flour-amaranth blends also revealed decreasing in L* value and increase in a* and b* values with increase in barrel temperature (Ilo *et al*., 1999). Increasing extrusion temperature will have a positive effect on texture, but at the same time initiate darkening of the color due to Maillard reaction. In order to get a good product one needs to strike a balance in obtaining appropriate texture and color with reference to process temperature.

The color evaluation of extruded products from wheat semolina and wheat semolina-fish mince mixture revealed a marginal increase in L* value with increase in barrel temperature. The products obtained from wheat semolina - ribbonfish (20%) mince at a barrel temperature of 90^{o}C gave an L* value of 77, whereas bull’s eye fish mince at 20% level under identical condition gave an L* value of 66. Under identical process temperature of extrusion the ribbonfish mince – semolina mixture was found to be better than that of bull’s eye fish mince-wheat semolina mixture, in terms of color. It is thus evident from the L* value (lightness of the product) is dependent on the type of fish used apart from barrel temperature. In the color space as described by Lauro (2000), a* and b* are the chromaticity coordinates, where a* is the red/green axis (‘+’ being toward the red and ‘-’ being toward the green). Similarly b* is the yellow/blue axis where ‘+’ being toward the yellow and ‘-’ being toward the blue. The a* and b* values obtained from wheat semolina-fish mince based products revealed that higher the barrel temperature higher the a* and b* value for control samples whereas incorporation of ribbonfish/bull’s eye fish mince did not follow a particular pattern. For instance addition of 10% ribbonfish had a lower a* value at a barrel temperature of 120^{o}C than at 70^{o}C and 90^{o}C. The changes in b* values with addition of fish mince to wheat semolina was marginal at different temperatures studied. Overall the results clearly indicate extrusion of wheat semolina-fish mince mixture did not significantly darken the color of the product, possibly due to the initial color of semolina which itself was slightly dark compared to rice flour.

The L*, a* and b* values of extruded products from ragi flour and ragi flour-fish mince mixture indicated relatively lower L* values and higher a* and b* values in comparison to products from rice flour. The changes in L* values as a function of barrel temperature with ragi based extruded products was not significant ($P<0.05$). This is because the color of ragi flour prior to extrusion was dark and any further darkening as affected by barrel temperature may not be significant in nature. The dark color of the extruded products from ragi is evident from the photographs (Fig. 28). The difference in b* values were not significant with respect to barrel temperature. However, addition of 10% fish mince caused significant ($P<0.05$) difference in Hunter a* and b* values (Table 25 C).

As a function of screw speed

Color analysis of extruded products prepared from a mixture of rice flour/wheat semolina/ragi flour and 10% ribbonfish mince at a constant barrel temperature of 90^{o}C using a die diameter of 4 mm revealed that the L* values of the products increased with an increase in the screw speed except for the product from rice flour with 10% ribbonfish mince (Table 26 A). At higher screw speed L* values increased, indicating that the sample was lighter (less cooked) (Apruzzese *et al*., 2000). As screw speed affects residence time and so would be expected to affect L* value (Apruzzese *et al*., 2000). Addition of fish mince caused significant ($P<0.05$) increase in L* values of rice flour based products and decrease in ragi flour based products (Table 26 B).

The a* values of extruded products prepared as a function of screw speed showed that the products which were having higher L* values showed lower a* values. In case of rice flour and wheat semolina based products a* values decreased with increase in screw speed, while that of ragi flour showed an increasing trend. Hunters b* values of extruded products from rice flour based products showed a decreasing trend with increase in screw speed (Fig. 41). In case of wheat semolina based products b* value increased with the screw speed up to 400 RPM and then decreased when screw speed reached 500 RPM. Ragi based extruded products showed increasing b* value with increase in screw speed. With increase in screw speed the color of rice flour base products were better than wheat semolina or ragi flour based products. As discussed earlier the initial color of the flour itself will have greater impact on the final color quality in addition to the interaction of added fish mince with the flour to alter the color complex of the final products.

As a function of die diameter

The effect of die diameter on the color of extruded products revealed an increase in L* value with increase in die diameter for wheat semolina and ragi flour based products. The products obtained from rice flour gave a higher L* value with a die diameter of 4 mm and further increase in die diameter reduced the L* value. The products obtained from wheat semolina and ragi flour with 10% ribbonfish mince showed lower L* values than the control at any screw speed attempted. In case of rice flour-ribbonfish mince extrudates, at a die diameter of 4 mm the maximum L* value of 76.58 was recorded indicating the extent of light color in the product. It is evident that increase in die diameter could increase the L* value for wheat semolina and ragi flour based products whereas for rice it decreased at a die diameter beyond 4 mm. This could be related to the impact of shear in relation to die diameter prior to expansion. The increase in L* value indicates that the product was lighter (not fully cooked) (Apruzzese *et al.*, 2000). This may be due to the lower pressure developed in the die end due to the bigger die size. Hunter a* value for rice flour based extruded product differed significantly as a function of die diameter. Hunters a* value of these extruded products from rice flour and wheat semolina decreased with increase in die diameter while that of ragi flour based products showed an increasing trend. The trend for Hunters b* value was also same for these products.

5.7.6. Texture analysis of extruded products

The texture of extruded products were analysed for crispness and breaking strength using texture analyzer.

5.7.6.1. Crispness

Crispiness of extruded products were carried out for samples extruded at a barrel temperatures of 70^{o}C and 90^{o}C, screw speed of 350 RPM and a die diameter of 4 mm. Both ribbonfish and bull’s eye fish (10% and 20%) along with rice flour/wheat semolina/ragi flour were used for extrusion process. Texture analysis of extruded products from rice flour, wheat semolina and ragi flour showed an increase in crispiness with process temperature. The number of peaks in extruded products from rice flour was more for the products processed at 90^{o}C irrespective of the percentage and type of fish mince used. The number of major peaks is considered as an indication of 'Crispness' (GRAMS/AI Software provided with TA-XT2 Texture analyzer). Extruded products from rice flours expanded to a greater level, this may be the reason for their crispy nature. Products from wheat semolina also showed the same trend, but there was less crispy for products with 20% fish mince irrespective of the barrel temperature. Increase in fish mince percentage cause slight increase in moisture content of the product and reduces crispiness. In the extrusion of corn starches, Duizer *et al*. (1998) reported that the products with low moisture content were crisper than those at higher moisture content. Addition of more meat will cause stronger interaction of flour-fish meat mixture and may lead to formation of stronger bonds, which will make the product tough. In the extrusion of potato starch-meat mixture, a protein gel was formed through aggregation of the meat soluble proteins during extrusion and was stabilized by intermolecular disulfide bonds (Moraru *et al*., 2002; Smith, 1988; Fennema, 1996; Matsumura and Mori, 1996). The crispness of the extruded products from ragi-fish mince was almost absent irrespective of the percentage of fish mince or barrel temperature used for extrusion. This was evident from the fracturability curve (Fig. 49&50) wherein the curve was smooth. One the other hand the curve obtained for the products from rice flour-fish mince showed number of small peaks indicating higher crispiness. The reason for lower crispiness in ragi flour-fish mince product is due to low expansion ratio.

5.7.6.2. Breaking strength

Breaking strength of all the extruded products with respective control were analysed as a function of barrel temperature, screw speed and die diameter.

As a function of barrel temperature

Extruded products from rice flour showed lesser breaking strength for products with 10% ribbonfish/bull's eye mince than that of the control samples. With increase in barrel temperature there was a significant reduction in the breaking strength of the extruded products obtained from rice flour-ribbonfish mince mixture (Fig. 51). At 10% level of fish mince the breaking strength was much lower than the products prepared from flour alone. The higher breaking strength of the products with 20% fish mince may be due to their higher moisture content. Ang *et al*. (1989) reported that the extrusion of rice-based products with 18% moisture yielded a hard textured product with incomplete gelatinization of starch. Addition of a protein source to a flour sample will definitely increase the breaking strength of the product. Noguchi *et al*. (1981) showed that the addition of soy protein isolate to rice flour made the extrudate more dense and rigid. In the extrusion process of fish mince-rice flour mixture, fish muscle acted more like filler and the expanded matrix was provided by starch in rice flour (Choudhury and Gautam, 2003). The breaking strength increased substantially upon addition of fish solids (Choudhury and Gautam, 2003). The value for rice flour extrudate was 104.84 kPa and with 15% fish solids, it increased to 246.38 kPa. Similar results were obtained during extrusion with fish-rice flour mixture (Gogoi *et al.*, 1996a, 1996b; Choudhury *et al.*, 1998). These results agree with the general observation of Areas (1992) that the addition of protein to starch rich flours produces the usual ‘protein-type’ extrudates that are harder, expand less and are more resistant to water dispersion.

The reduction in breaking strength of wheat semolina based products with increase in barrel temperature was not significant ($P<0.05$). Wheat semolina with 20% ribbonfish at a barrel temperature of $70^{o}C$ required higher breaking strength for its fracture (Table 32 A). Even low levels of moisture can significantly affect deformation properties and texture due to changes in the distribution of fracture intensities (Barrett and Kaletunc, 1998). With 10% fish mince the breaking strength of the product was lower than that of the control products. The addition of ribbonfish mince caused considerable increase in breaking strength of wheat semolina based products. The products with 20% fish mince expanded less compared to other samples. This may be another reason for their higher breaking strength. Owusu-Ansah *et al.* (1984), Bhattacharya and Hanna (1987) and Chinnaswamy and Hanna (1988) pointed that the higher the radial expansion, the lower was the reported shear stress (breaking strength).

Breaking strength of ragi based extruded products also decreased with increase in barrel temperature (Fig. 51). The breaking strength of the control samples was the least among all the extruded products. The addition of 10% ribbonfish mince cause significant ($P<0.05$) increase in breaking strength of ragi based extruded products (Table 32 B).

As a function of screw speed

Extruded products prepared from a mixture of rice flour/wheat semolina/ragi flour and 10% ribbonfish mince at a constant barrel temperature of $90^{o}C$ using a 4 mm die diameter showed an increase in breaking strength with increase in screw speed. Addition of fish mince caused significant ($P<0.05$) increase in breaking strength of wheat semolina based extruded products (Table 33 B). There will be reduction in residence time with increase in screw speed. Thus the degree of gelatinization will vary with increase in screw speed. Probably this may be the reason for increase of breaking strength with increase in the screw speed. Breaking strength of the control samples from ragi flour showed the least values.

As a function of die diameter

Breaking strength of extruded products from rice flour/wheat semolina/ragi flour with 10% ribbonfish mince prepared at a constant barrel temperature of 90°C and screw speed of 350 RPM showed an increasing trend with an increase in die diameter. However, the increase was significant ($P<0.05$) for wheat semolina based extruded products only. Here also the ragi control samples showed the least values. With increase in die diameter, the pressure developed in the die head will reduce and hence the expansion ratio of the product was also less. This may be the reason for the higher breaking strength for the products prepared using 5 mm die diameter compared to that of 3 mm die diameter. Addition of fish mince did not show any significant ($P<0.05$) change in breaking strength of extruded products prepared as a function of die diameter (Table 34 B).

Considering the different quality attributes of extruded products as discussed above it is evident that good quality extruded products can be prepared using rice flour-ribbonfish at 10% level with extrusion processing conditions like barrel temperature of 90°C and die diameter of 4 mm. Quality analysis of extruded products from wheat semolina-fish mince mixture revealed that better products can be prepared using wheat semolina with 10% bull’s eye fish mince at a barrel temperature of 120°C and screw speed of 350 RPM using a 4 mm die diameter. Optimum extrusion variables for ragi based extruded products were a barrel temperature of 90°C and screw speed of 350 RPM using a 4 mm die diameter. The most compatible fish species for ragi flour based extruded products was found to be bull’s eye fish mince at a concentration of 10%.

5.8. Gelatin

Gelatin is a food protein and used as an ingredient to improve consistency and stability of various food products. Apart from this food gelatin has a broad application in pharmaceuticals, photography and as packaging material. In the present investigation gelatin from skin of bull’s eye fish (*Priacanthus spp.*) have been prepared and its physicochemical and rheological properties were assessed. The properties of gelatin were assessed using freeze-dried sample wherein the moisture content was less than 3%.

5.8.1. Proximate composition of bull's eye fish skin

The protein content in skin of bull's eye fish was found to be 25.19%. The fat and ash contents were 1.24% and 20.23% respectively (Table 35). Since the fat content is low it can be easily removed during extraction.

5.8.2. Yield

The yield obtained for the extraction of gelatin in the present investigation was found to be 3.9 %. The yield obtained for the extraction of gelatin from hake (*Merluccius merluccius*) was 6.5% (Gomez-Guillen *et al.*, 2002). Grossman and Bergman (1992) and Gudmundsson and Hafsteinsson (1997) reported gelatin yield of about 15% for tilapia and cod respectively. The lower yield recorded in this experiment could be due to the leaching of collagen during the washing treatments. Since washing with water was carried out 3 times after each treatment to achieve neutral pH, some of the collagen in the skins may have leached out during draining (Jamilah and Harvinder, 2002).

5.8.3. Properties of freeze dried gelatin

The protein content of freeze-dried gelatin was 94.6% and more than 90% was soluble in phosphate buffer containing 0.3 M NaCl (pH: 7.5). After freeze-drying the moisture content of the gelatin was 2.2%. Bloom strength values of the gelatin prepared in the present investigation recorded a value of 108 g. The bloom strength values of gelatin obtained from skin of hake, cod and megrim varied from 100 g to 210 g (Montero and Gomez-Guillen, 2000; Fernandez-Diaz *et al.*, 2001; Gundmundsson and Hafsteinsson, 1997). The bloom strength of gelatin from the skin of bull's eye fish was slightly on the lower side compared to that of cod and hake. Gomez-Guillen *et al*. (2002) reported that gelatin from the skin of soles gave the highest bloom strength values of nearly 350 g. The gel strength of gelatin extracted from fish skin is comparatively lower than that of porcine gelatin. Choi and Regeinstein (2000) found that the gel strength value of gelatin prepared from fish skin was lower whereas porcine gelatin recorded a value of 300g. It has been observed that the proportion of hydroxyproline in gelatin is responsible for lower bloom strength (Arnesen and Gildberg, 2002).

5.8.4. Aminoacid composition of gelatin

Aminoacid composition of gelatin prepared from skin of bull's eye fish showed higher proportion of glycine (27.16%). Aminoacid analysis of gelatin from tilapia, horse mackerel and cod showed a higher proportion of glycine (more than 30%), which is the most dominant aminoacid in gelatin (Arnesen and Gildberg, 2002). The imino acids (Pro + HyP) content of the gelatin prepared from bull's eye fish skin was found to be 18.43%. The aminoacid analysis of gelatin prepared from different fishes like cod, hake, megrim and sole showed around 30% glycine and 17% imino acids (Pro + HyP) (Gomez-Guillen *et al.*, 2002). The stability of triple helical structure in renatured gelatins has been reported to be proportional to the total content in pyrrolidine imino acids, given that it is the Pro + HyP rich zones of the molecules that are most likely involved in the formation of nucleation zones (Ledward, 1986). However, although Proline is important, Hydroxyproline is believed to play a singular role in the stabilization of the triple – stranded collagen helix due to its hydrogen bonding ability through its –OH groups (Burjandze, 1979; Ledward, 1986). The higher content of Proline, Hydroxyproline and Alanine in a commercial gelatin from tilapia has been shown as one of the major causes responsible for its higher viscoelastic properties when compared to a megrim gelatin (Sarabia *et al.*, 2000). Thermal stability of a gelatin gel has been shown to be directly correlated to the number and stability of Proline rich regions in the collagen or gelatin molecules, which in fact is considerably lower in cold-water fish than in warm-water fish (Ledward, 1986). Fish gelatin show a wider variation in composition, their hydroxyproline and to a lesser extent proline contents were lower than that of mammalian gelatin and this is compensated by higher concentrations of the other hydroxyamino acids *viz.* serine and threonine (Balian and Bowes, 1977).

5.8.5. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis of gelatin

SDS-PAGE pattern of gelatin from the skin of bull's eye fish showed two intense bands at molecular weight region of 120 KD and 95 KD respectively, which corresponds to α-chains and β-chains (Fig. 54). According to the values referred by Bourgeois and Roux (1982), the bands corresponding to molecular weight ~ 120 kD may be identified as α-chains and those with ~ 170 kD could be considered the β-chains. However, Gomez-Guillen *et al.* (2002) reported that the bands corresponding to 95 kDa and 200 kDa in the

SDS PAGE of gelatin represents the collagen α- chains and β components (Gomez-Guillen *et al*., 2002).

5.8.6. Setting index of gelatin at different concentrations

Setting index of gelatin showed that with increase in concentration of gelatin solution, time taken for complete solidification reduced (Fig. 55). The setting index is a macroscopic property wherein solidification process is observed visually and the methodology is highly subjective. Choi and Regenstein (2000) observed that with increase in concentration the gel strength of gelatin increased. The gel strength of gelatin gels rose sharply with increased maturation time up to 4 hours. This increase in gel strength has been attributed to the fact that each polypeptide chain of gelatin becomes ordered, either by the growth of existing junctions or by the formation of new but less stable junctions from the regions containing a lower content of pyrrolidine residues (Ledward, 1986). In the present study the setting index indicated gelatin with 40 mg/ml took a time of 105 minutes for complete solidification. On the other hand at a concentration of 10 mg/ml the time taken for solidification was 180 minutes. This demonstrates formation of ordered structure is dependent on concentration as reported by several researchers.

5.8.7. Rheological properties of gelatin solution

The flow profile of the proteins at specific temperature provides information on resistance to shearing thereby indicating structural impairment, if any. Irrespective of the concentration of sample at temperatures of 25^{o}C, 40^{o}C, 50^{o}C and 60^{o}C, shearing caused the impairment of the sample (Fig. 57&58). The flow behaviour of gelatin solution showed that at 40^{o}C and 50^{o}C structural impairment occurred as evident from the down curve data. At 10^{o}C it showed minimum thixotropic area (Fig. 59). The flow profile data of gelatin samples as a function of concentration and temperature indicated non-Newtonian behavior. Further, shear-thinning phenomenon was evident wherein increase in shear rate decreased the viscosity. The flow profile carried out at 10^{o}C showed minimum thixotropic area indicating minimum damage to the structure of gelatin due to shearing. The change in structure could be either a build up (in which the process is called 'Rheopexy' or 'Antithixotrophy') or a breakdown. It can also be the overall result of both processes occurring at once, that is shearing cause structure to breakdown the sample, but at the same time, the natural behavior of the sample is to build-up structure. Hence the two processes occur simultaneously and the overall picture is dictated by the relative ratio of the two (Barnes *et al*., 1989). At higher concentration of gelatin (30 mg/ml), the flow profile data indicated coagulation at higher temperature.

VI. SUMMARY

With increase demand for fish and fishery products World over, supply of acceptable quality fish to increasing population is being accorded top priority by planners. About 20-25% of total fish catch is lost due to spoilage because of its inherent characteristics and also due to lack of required infrastructure for its preservation. While several species of by-catch, non-conventional and underutilized species are equally important from nutritional point of view; its utilization in fresh form is being limited because of poor consumer acceptance. In order to utilize by-catches, which are normally of low economic value, attempts are being made for the development of novel processing techniques so as to reach the consumer at affordable prices. Extrusion processing is one such technology where it is being expanded to fish mince based cereal products so as to achieve supply of nutritious food. Extruder plays several important functions in the processing of these foods. Among the most important are precooking and gelatinization of the starch, providing a desired shape to the product and giving the product an expanded, crisp and pleasing characteristics. Addition of fish meat to cereal based extruded products will proportionally add to the nutritive value. Utilization of low economic value fish for extrusion processing is another step stone in managing the optimum utilization of under utilized fish and by-catches.

Utilization of fish processing waste like skin and bones is receiving attention by various researchers with twin objectives avoiding environmental pollution and to derive value added products for better economic viability. Gelatin is one such product, which can be obtained from skin of various marine fish species. The conventional source of raw material for gelatin preparation is from the skin of bovine and porcine sources. With the recent outbreak of mad cow disease (Bovine Spongiformes Encephalopathy) the industry is looking for alternate raw material for gelatin preparation.

With this rationale the objective of the present investigation was to

- Utilize low economic value fish like ribbonfish and bull’s eye fish for extrusion process with cereal flours (rice flour, wheat semolina and ragi flour) using twin-screw extruder.

- To characterize fish proteins and starch from cereal flours used for extrusion process by thermal and optical analysis.
- To optimize the process variables like barrel temperature, screw speed and die diameter of the extruder and the quantity of fish meat.
- To assess the composition and properties of fish mince-cereal based extruded product.
- To prepare and assess the properties of gelatin from skin of bull's eye fish.

Ribbonfish (*Trichiurus spp*.) and bull's eye fish (*Priacanthus spp.*) caught by trawl along West coast off Mangalore were used in the present study. Fish mince without skin, scales and bones were used for the extrusion process. The fish mince was mixed with individual cereal flours either at 10% or 20% and after proper preconditioning subjected to extrusion process. Both the fish belong to low fat fish having fat content of below 2%. The protein content of ribbonfish and bull's eye fish meat was 17.91% and 16.71% respectively. The amino acid profile revealed that both the fish were having a higher proportion of alanine, glutamic acid, lysine and leucine. The lysine content of ribbonfish and bull's eye fish meat was 10.57 and 9.79 (as percentage amino acids) respectively. The fatty acid profile revealed that the major fatty acid present in ribbonfish was erucic acid or cetoleic acid (C22 : 1n9) and that of bull's eye fish were heptadecanoic acid (C17 : 0) and arachidic acid (C20 : 0). The EPA and DHA content of ribbonfish and bull's eye fish were 11.83 & 7.86 and 6.99 & 8.96% respectively.

The protein content in wheat semolina (11.88%) was higher than rice and ragi flour. Higher ash content in ragi showed the presence of high amount of calcium and soluble fibers.

The thermogram (DSC profile) of the meat from both the fish showed three transitions. For ribbonfish meat, the peak temperatures for the denaturation of myosin, sarcoplasmic protein and actin were at 33.17^{o}C, 48.85^{o}C and 60.96^{o}C and the corresponding transitions for bull's eye fish meat were at 38.35^{o}C, 47.72^{o}C and 63.02^{o}C. The DSC profile of flours/semolina revealed that the peak gelatinization temperature of rice flour, wheat semolina and ragi flour were at 64.73^{o}C, 58.16^{o}C and 69.72^{o}C respectively.

FT-Raman spectra of proteins from ribbon and bull’s eye fish showed a strong band at 1005 cm^{-1} indicating phenylalanine compound. The sharp peak at 760 cm^{-1} in both ribbonfish and bull’s eye fish is due to indole ring of tryptophan. The ratio of the bands I_{855}/I_{830} for ribbonfish was 0.64 and that of bulls eye was 0.80, indicating that tyrosine residues are not exposed.

The FT-Raman spectra of native rice flour, wheat semolina and ragi flour revealed a conspicuous peak at a wavenumber of 480 cm-1 due to presence of α-(1-4)-glycosidic linkage. In the spectra of wheat semolina there was two additional peaks between 1663-1630 cm-1 and another between 1595-1528 cm-1, which corresponds to Amide I and Amide II respectively. In wheat semolina and ragi flour after gelatinization the intensity of all bands decreased except the bands at 1633 cm-1 and 3213 cm-1, which were increased indicating the irreversible absorption of water. In the spectra of rice flour the spectral changes after gelatinization was marginal.

Dynamic rheological properties of flour samples (10% w/v solution) revealed that the maximum G’ (storage modulus) for rice flour, wheat semolina and ragi flour were 6029 Pa, 118 Pa and 544 Pa respectively. The temperature at which cross over (gelling point) has taken place for rice flour, wheat semolina and ragi flour during dynamic rheological testing was found to be at 61.63^{o}C, 60.22^{o}C and 52.68^{o}C respectively.

Phase contrast microscopy revealed that the shape of starch granules was irregular varying from round to polyhedral. All the granules were found to be intact and there was no disruption in shape. In the present investigation phase contrast microscopy revealed that the granule size of wheat semolina was higher than rice or ragi flour. The phase contrast microscopic photograph of the flours in wet state showed swelling of the granules. After gelatinization the granules swell to a greater extent and the swelling was greater for wheat semolina as compared to rice and ragi flour.

Extruded products

Extruded products were prepared from a mixture of rice flour, wheat semolina and ragi flour with and without addition of fish meat (ribbonfish/bull’s eye fish) as a function of barrel temperature, screw speed and die diameter. As a function of screw speed and die diameter, samples were prepared only with ribbonfish meat at a concentration of 10% and their respective controls.

The moisture content of all the extruded products decreased with increase in barrel temperature, indicating higher temperature during extrusion process could reduce the moisture content of final product. However with increase in fish mince content, higher moisture content was recorded in the final product.

With increase in screw speed the moisture content of the products showed an increasing trend. This reveals that residence time will be shortened with increase in screw speed, hence the moisture content of final products increased.

The moisture content of all the extruded products increased with increase in die diameter. This is mainly related to the pressure developed inside the barrel wherein, it progressively reduces with increasing die diameter.

The protein content of the extruded products did not show any significant difference with respect to variables such as barrel temperature, screw speed and die diameter. However, the fat content did show some variation as the function of different variables and was related to moisture content.

Aminoacid composition of extruded products from a mixture of rice flour-fish mince (ribbonfish and bull’s eye fish meat) mixture showed a higher proportion of glutamic acid, alanine, tryptophan, leucine and lysine. With increase in percentage of fish meat from 10% to 20%, the percentage of essential aminoacids especially lysine increased.

Expansion ratio

The expansion ratio of extruded products from flour-fish mince (ribbonfish/bull’s eye fish) increased with increase in barrel temperature. The highest expansion ratio of 375% was obtained for the products extruded at 120oC with rice flour and ribbonfish at 10% level. Products with ragi flour-fish mince mixture showed the least expansion ratio at different temperatures tried.

The effect of screw speed on the expansion ration of extruded products revealed no significant difference between control and fish mince based products. The extent of decrease in expansion ratio in relation to screw speed was higher in samples incorporated with fish mince.

The expansion ratio of different extruded products as a function of die diameter indicated a decreasing trend with increase in die diameter. The extent of decrease was minimum in case of ragi flour and ribbonfish mixed extrudates. This could be related to higher moisture content and improper gelatinization in the final product.

Water absorption capacity (WAC)

The water absorption capacity of the extruded products increased with increase in barrel temperature. At 10% fish mince incorporation (either ribbonfish or bull's eye fish) to rice flour yielded a water absorption capacity of 7.0 g water/g dried material. When the fish meat proportion was increased to 20% there was significant decrease in WAC values in all the samples.

The increase in WAC of extruded products as a function of screw speed revealed divergent results for different products. For wheat semolina-10% ribbonfish mince significant increase in WAC values with screw speed was recorded. WAC values of products with rice or ragi flour and fish mince recorded a marginal increase. The WAC values of samples are mainly dependent on the nature of protein and the extent of exposure of hydrophilic group upon processing.

The increase in WAC values with incorporation of 10% ribbonfish mince was more in case of rice flour than with wheat semolina and ragi flour products. Wheat semolina – fish mince based products registered a decrease in WAC value with increase in die diameter for which no convincing reason could be given.

Colour

The color of the extruded products was assessed by Hunters colorimeter with L*, a* and b* co-ordinates. Higher L* values indicates lighter color of the product. The L* values of the products obtained from rice flour only (control) showed a decreasing trend with increase in temperature indicating darkening of the products. However, the L* values of the products obtained from wheat semolina and ragi flour revealed an increase in L* value with increase in barrel temperature. This could be attributed to the reactions in the pigments of the respective flours with increase in temperature. Incorporation of fish mince at 10% level could significantly increase the L* values with increase in temperature in all the extruded products. There was an inverse relationship between L* and a* or b* values in all the samples studied. The color of the ragi flour based extruded products with respect to L* values were consistently lower compared to rice flour or wheat semolina products.

The L* values of the control samples (without fish meat) obtained from different flours revealed increasing trend with increase in screw speed. However this increase was not significant. The extrudates from rice flour and 10% ribbonfish mince showed a significant increase in L* value as a function of screw speed over that of respective control. The extrudates obtained from mixture of wheat semolina-10% ribbonfish mince and ragi flour-10% ribbonfish mince registered a decreasing L* value with increase in screw speed. Thus it is evident that addition of ribbonfish mince to wheat semolina and ragi flour registered a dark color with increase in screw speed while the extruded products with rice flour-10% ribbonfish mince had a light color.

The L*, a* and b* values of extruded products obtained as a function of die diameter did not show any appreciable change. From the results it is evident that barrel temperature and screw speed had influenced the development of desired color.

Texture analysis of extruded products

The texture of extruded products with respect to crispness and breaking strength has been analyzed. The products prepared with 10% fish mince (ribbonfish/bull's eye fish) at 90oC were crispier irrespective of the flour or fish mince. Higher crispness was recorded in the extruded products obtained from rice flour – fish mince and wheat semolina – fish mince as compared to ragi flour – fish mince. The crispness was related to expansion ratio of the product and as products from ragi flour had lesser expansion, the product was less crispy. The crispness was assessed only with products prepared at a screw speed of 350 RPM and die diameter of 4 mm.

The breaking strength of the extruded products obtained from flour/semolina (control samples) decreased with increase in barrel temperature. Addition of 10% ribbonfish or bull's eye fish to the flour/semolina did not affect breaking strength significantly as a function of temperature. When the fish meat proportion was increased to 20% higher breaking strength was recorded in all the extrudates especially when the barrel temperature was 70oC. Breaking strength is an indicator of the force required for fracture and higher breaking strength means improper gelatinization and lesser expansion ratio. An inverse relation could be focused between crispness and breaking strength values obtained under identical conditions.

The effect of screw speed on the breaking strength of the extrudates obtained from rice flour-fish mince and ragi flour – fish mince showed an increasing trend with increase in screw speed. However wheat semolina-fish mince mixture the breaking strength reduced considerably as compared to the control at any screw speed in the experimental regime. Though explanation could be found with reference to rice and ragi extrudates with respect to improper gelatinization, the same could not be true with wheat semolina based extrudates. This could be perhaps due to behaviour of gluten in wheat to different shear condition as induced by screw speed.

The effect of different die diameter in extrusion process on the breaking strength of extrudates revealed ragi flour- fish mince mixture had a significant effect in increasing the breaking strength of the final products.

The study revealed that good quality extruded products can be prepared using rice flour-ribbonfish at 10% level with extrusion processing conditions like barrel temperature of 90oC and die diameter of 4 mm. Quality analysis of extruded products from wheat semolina-fish mince mixture revealed that better products can be prepared using wheat semolina with 10% bull’s eye fish mince at a barrel temperature of 120oC and screw speed of 350 RPM using a 4 mm die diameter. Optimum extrusion variables for ragi based extruded products were a barrel temperature of 90oC and screw speed of 350 RPM using a 4 mm die diameter. The most compatible fish species for ragi flour based extruded products was found to be bull’s eye fish mince at a concentration of 10%.

Gelatin

Gelatin has been prepared from the skin of bull’s eye fish (Priacanthus spp.) and its physicochemical and rheological properties were assessed. The proximate composition of bull’s eye fish skin showed that the moisture content was 52.79% and a protein content of 25.19%. The yield of gelatin obtained in the present investigation was found to be 3-4%.

The protein content of freeze-dried gelatin was found to be 94.6% and 93% of protein was found to be soluble in phosphate buffer containing 0.3 M NaCl. The moisture content of freeze-dried gelatin was 2.2%. Bloom strength values of the gelatin prepared recorded a value of 108 g.

The aminoacid composition of gelatin from the skin of bull’s eye fish revealed higher proportion of glycine. The other major aminoacids present in the gelatin were alanine (19.45 %) and hydroxyproline (13.45%).

SDS PAGE pattern revealed the presence of bands at 120 KD and 170 KD revealing the presence of α-chains and β-chains respectively.

Setting index data revealed that with increase in concentration of gelatin solution, time taken for complete solidification reduced considerably. At a concentration of 10 mg/ml 100% solidification occurred after 180 minutes; when the concentration was increased to 40%, time taken for complete solidification was 105 minutes.

Flow profile of gelatin solution of various concentrations as a function of temperature has been assessed. Irrespective of the concentration of sample at temperatures of 25^{o}C, 40^{o}C, 50^{o}C and 60^{o}C, shearing caused the impairment of the sample as evident from the down curve data. Flow profile of gelatin solution (30 mg/ml) at 10^{o}C showed minimum thixotropic area between up curve and down curve indicating least damage to the structure as affected by shearing. The flow profile data of gelatin solution indicated non-Newtonian behavior with pseudoplastic behavior at all concentrations and temperatures.

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