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 up to 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°-67.6°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°C; midpoint, 61°C and end point, 64°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°C-60°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 photopasteographs. Miller et al. (1973) also used photomicrographs, 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 Meacham, 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°C to 70°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 an 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 Haism, 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, $\delta$) 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-dimensional 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°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 45º-90ºC, 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° and 73°C 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 amylpectin 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, amyllopectin 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°C) and normal low $T_p$ starches (62.8°-67.0°C), intermediate $T_p$ starches (71.7-73.5°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°-200°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°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 $\Delta H$ 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 $\Delta H$ measured. When the sample was 100% damaged, $\Delta H$ 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°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° to 200°C) 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; Wiriyawepaiwong et al., 2004).

The temperatures reached by the food during cooking extrusion can be quite high (200°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 ⁶⁴Cu. 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 amyllopectin 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 70º to 120ºC 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 60º, 90º, 125º, 150º and 190ºC. They found that when the extrusion temperature was increased to 125ºC, 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°-170°C 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°C. The temperatures for the second stage were varied from 60° to 140°C. After extrusion the product was cut into pieces and then dried in a forced-air cabinet drier at 70°C for 6 hours to give final moisture content of 8-9%. For the product extruded at temperature of 100°C 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°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 l’ 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°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° 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-bean 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 Galazka, 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 Mulvayne, 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°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).