CHAPTER - III

Surface Microstructural Features of Common Edibles.
Introduction

The use of scanning electron microscope in the determination of structural changes in the wide varieties of human edibles is finding increasing applications (Carrol & Jones 1979). Many food processing operations are designed to create the microstructure that gives the food products their characteristic properties. As for example, from milk one can produce different types of cheeses, yogurts, spreads, butter, cream, ghee or whipped products, where the properties are determined by the structures. Other examples of fabricated foods are cereal products such as pasta, meat products, e.g., sausages and so on. Colloidal structures like gels, emulsions, foams and other combinations determine the type of these products. How a particular structure is engineered, and therefore how it relates to the properties of the product can be described with the help of electron microscopy (Hermannson et.al., 2000).

Most food consists of components in the form of particles large enough (>1μm) to be visible under light microscope. Optical microscopy makes it possible to stain the components and thus characterize them by colour. Scanning and transmission electron microscopy (SEM & TEM) increases the resolution of traditional optical microscopy and markedly increases the depth of focus (Kalab 2000). Electron microscopy provides much greater details than optical microscopy. However, since in electron microscope no light but a focused beam of electron is used to enlarge the image of the sample, the resulting micrograph cannot be in colour, but they consist of various shades of gray. Individual components are distinguished through differences in their affinity for various heavy metals such as osmium, lead, ruthenium and uranium (Kalab 2000).
To understand the structural role of individual components and their effect on the overall microstructure of complex food structures, microscopy in combination with other techniques can help. There are a number of microscopy techniques available, and each technique provides part of the information necessary for a full understanding of the microstructure of foods and other complex bio-meters (Hermannson et.al., 2000).

Several microscopic techniques are required to cover this range and different routes of preparations enable the scientists to focus on specific information, such as interactions between bio-polymers, interfacial structures and bulk structures. The effect of change in one bio-polymer within a complex assembly and to follow, how a change can effect the behaviour of the overall structure can be studied by combining several techniques. This provides a tool for exploring the biophysical reasons behind relationship between structure and behaviour such as mechanical properties.

New avenue for research in the field have been opened up with new developments in the field of microscopy like digitization of images and the computer power to process image information.

The relatively great depth of focus and high resolution makes the scanning electron microscope an excellent tool in studying the structure of foods, even those which consists of large components, like meat products or baking products. The characteristic scanning electron microscopic property of showing only surface is no hindrance since new surfaces may be produced by fracturing the sample. For example, yogurt is very interesting from the structural point of view, because it has high water content and yet is solid. Electron microscopy reveals some peculiarities in the development of yogurt structure. The changes which take place in milk when yogurt is formed, involve particles
so small that they cannot be seen by light microscope. Milk placed under optical microscope reveals only fat globules. Electron microscopy, however, shows the prevalent milk protein i.e., casein, as minute globules about 100 nanometer in diameter, which are called ‘casein micelles’ evenly dispersed in the milk. Electron microscopy makes it possible even to study the surface of casein micelles. In fresh milk they remain separated from each other, and the surfaces of the casein micelles are non-reactive. This situation changes however, when the casein micelles are destabilized, such as by the introduction of lactic acid in the system. This whole process of change can be observed and understood using electron microscopy (Kalab 2000).

Scanning electron microscopy and x-ray micro analysis were also used to conduct forensic investigations on glass and foreign objects in various food products. As for example, glass fragments, insects, reptile’s and rodent’s body parts etc. metal foreign objects from various sources can easily be distinguished using this method (Charbonneau 2001). Scanning electron microscopy has also been used to study and compare the structural characteristics of stored food products in various temperatures, like ambient conditions or refrigerated conditions (Berrios et.al., 1998).

The scanning electron microscope was introduced commercially as a scientific instrument in the year 1965 (Voyle 1981). The use of scanning electron microscope in the determination of structural changes in a wide verities of food and food products such as meat, is finding increasing applications. (Carrol & Jones 1979).

An important desired characteristic of meat is tenderness, a quality easy to determine at meal time but difficult to predict by any objective measurement. Much
information is now available concerning the factors contributing to toughness or tenderness of meat (Carrol & Jones 1979).

Thick and thin muscle filaments overlap sliding past each other during the process of contraction and relaxation, which is widely studied by several workers (Hauson & Huxlay 1955; Voyle 1979; Voyle 1981). Skeletal muscle from rainbow trout, turkey and beef were examined by Schaller and Powrie (1971).

Changes in chemistry and morphology of muscle are initiated at death with the process of glycolysis and the consequent fall of pH. These changes contribute to the process known as 'aging'. Protein which are denatured, become exposed to the proteolytic activity of endogenous enzymes. This has an effect on the texture of the tissue as meat, an effect which can be followed by microscopic observations of the changes in the morphology of the muscle fibre. Changes can be seen in sercoplasmic reticulum (Scheller & Powrie 1971), the sercolemma (Varriano-Maston et.al., 1976) and the myofibrils (Davey & Dickson 1970; Sayre 1970; Stanley 1974). Sercoplasmic reticulum apparently deteriorates while aging or storage. The location of transverse elements of sercoplasmic reticulum which was initially represented as ridges, changes its appearance to tough shape during post-mortem period (Scheller & Powrie 1971). This implies collapse of the tubular system of sercoplasmic reticulum or alternatively, the swelling of adjacent regions of the sercomare. The scanning electron microscope facilitates the detection of such changes more readily because of the three-dimensional appearance of the image (Voyle 1981). Also, breaks in myofibrils in the region of the transverse element take place (Davey & Dickson 1970; Seyre 1970).
Over a longer period of storage of meat, about 12 days, changes in the fine structure of sercolema of free and restrained muscle has been observed, during which structure degenerates from a relatively smooth membrane around the fibre, to a collection of randomly distributed aggregation of protein (Varrieno-Marston et al., 1976). Individual myofibrils are discernible beneath the aggregated layer. The implications of these changes with respect to meat texture are obvious and important, as are the changes in the structure of myofibrils (Voyle 1981).

Of all the treatment that can influence the texture of meat, cooking is probably the most important because it acts on all the tissue components, irrespective of their identity; myofibrilar proteins get coagulated, collagen shrinks and is converted into gelatin and water gets released. At the temperature of about 97°C myofibrils from bovine, rainbow trout and avian muscle showed loss of organized structure of the thin filament in the I-band. Besides this, the trout myofibrils heated at same temperature showed a gap at the location of H-zone (Schaller & Powrie 1971).

When the sercoplasmic protein gets denatured, the granular material is formed and fine collagen fibre become indistinguishable and larger fibres become swollen (Doty & Pierie 1961; Paul 1963).

All these examples suggest the versatility of microstructural studies in assessing food qualities.

Studies on food microstructure need an integrated approach because inconsistencies occur when transformations, interactions and properties at different levels of organization are compared and interpreted as one and the same.
The total system can be evaluated in terms of the overall properties of the product; at the particulate or component level and at the molecular and atomic level. Studies of food microstructure, regardless of the type of system being studied are therefore, not entities unto themselves but are simply portions of the information needed in an integration of data leading to a fuller understanding of the total system.

Food microstructural studies appear to be exciting because of an analytical and quantitative data can lead to several vital information regarding the quality and consumer acceptability of different types of food. Formulated food for example, are becoming more prevalent as food sources and it is imperative to understand the exact cause and effect of chemical and structural changes. As for example, chlorination of 'cake-flour' and possible alternate sources of treatment that result in similar flour functionality are important. Soy substitutions in 'meat-emulsions' and other systems poses special problems in terms of functionality, safety and sensory characteristics (Davis & Gordon 1980).

A review on the subject reveals that studies on food microstructure are restricted to some western countries. Texture and structural changes in foul meat due to different high temperature heat treatments and meat moisture contents were examined by the use of electron microscope and torsion analysis (Volter et.al., 1996). Berrios et.al., (1998) studied the structural characteristics of stored black-beans with the help of scanning electron microscope. Milk protein gelation and cheese melting was studied by Auty and coworkers (1999). Garcia and coworkers (1999) made an extensive study on edible starch films and coating characterization with the help of scanning electron microscopy. Microstructural changes in zein proteins during extrusion have been studied by
Batterman-Azcona et al., (1999). Microstructure of whole milk powder and the insolubles were detected by McKenna et al., (1999). Similarly morphological changes of starch granules in the apple-cultivars during ripening and storage were studied by Kovacs and Eads (1999). Cereal seed storage protein structures were determined by Tatham et al., (1999) with the help of scanning probe microscopy. Allan-Woztas et al., (1999) studied the effect of freezing methods and frozen storage conditions on the microstructure of wild berries as observed by cold-stage scanning electron microscope.

Foreign substances in food were investigated by Charbonneau (2001) with the help of scanning electron microscope. Structural changes in apple-rings during convection air-drying with controlled temperature and humidity were reported by Bai et al., (2002). Forster and coworkers (2002) studied the structure, physico-chemical properties and in-vitro fermentation of enzymetically degraded cell-wall materials from apples. Kalab (2000) made microstructural studies on soy foods, milk and its constituents.

All these studies indicate that investigations on food microstructure are extremely rare in our country as compared to other parts of the globe. This has certainly created a lacuna in our knowledge regarding food and its components. Although in other parts of the country some studies are carried out on different aspects of food, particularly in food chemistry (Srivastava et al., 1995; Mukhopadhaya et al., 1998; Rao & Agarwal 1999; Sidhu 2003; Tharanathan & Mahadevamma 2003), in the northeastern parts of India virtually no work has been done so far in understanding the food quality in terms of wholesomeness. Hence, any attempt in acquiring knowledge on some aspects of food
quality e.g., food microstructure appears to be relevant in context of this region in particular and India as a whole.

The present study has therefore been taken up on different aspects of food microstructure to understand the food quality of edibles consumed by people of this region.

It is known that Northeast India is geo-climatically different from other parts of the country (Seiler & Sigal 1988). This is likely to be responsible for positive as well as negative changes in chemical and structural characteristics of plants grown in this part. Hence, studies on microstructural features of some vegetables e.g., cabbage, cauliflower etc. were carried out to compare the hard and soft leafy edible portions.

Scanning electron microscopy of skin and seed of green chilly, dried green chilly, unripe red chilly and dry red chilly were taken up in view of popular belief on adverse affect of chilly on digestive system.

Curd which is prepared in this region had also never been investigated in terms of its quality. Hence, scanning electron microscopic studies on curd were taken up to asses its quality from structural features which also appear to be relevant.

Different types of meat are preferred by various communities residing in the Northeast region of India. Since the animals grow and develop by consuming plants of this region, whose nutrient qualities have not been studied in detail, virtually nothing is known about the quality of meat consumed by the people of this region. Microstructural studies appear to be important in this regard to asses the quality of meat to a considerable extent. Further, meat is usually preserved for a considerable length of time before consumption by the people here. In view of the earlier findings on the effect of low-
temperature storage of meat (Voyle 1981) it is felt that microstructural studies on meat with the help of electron microscope is needed in terms of freezing effect of different durations and effect of boiling.

As far as the fish consumption in this region is concerned, it is known that bulk of the fishes marketed here are from neighboring states. In some parts of the region e.g., Meghalaya very few local fishes are available in the market, and fishes which come from other places need a minimum of six hours to several days to reach the local markets. It is quite likely that a number of changes may take place in the fish during this transit. It is quite surprising that no attention has been paid in this regard by the researchers of this region. Keeping these in view surface microstructural studies involving scanning electron microscope have been carried out on fish muscles to asses the structural changes on post mortem effects.

Studies have also been conducted on some spices that are marketed from other parts of the country and those which are processed locally to understand their structural features and association of foreign matters if any. Structural features of bread (prepared locally) before and after toasting also appears to be relevant in terms of its qualitative assessment.

Different verities of edibles prepared from rice or paddy e.g., flattened-rice/paddy (chira), popped or expanded-rice/paddy (khai) and puffed-rice (muri) involving indigenous techniques are highly popular among people of this region. It was thought that scanning electron microscopy of surface features of these products will be extremely important in assessing their qualitative aspects from the structural features. It can also evaluate the indigenous methods of processing in terms of their scientific approaches.
Aims:

- To understand the surface microstructure of some common edibles.

- Microstructural changes of edibles during freezing.
Materials and Methods

Materials:

The materials used for microstructural studies involving scanning electron microscopy were:

1. **Brassica oleracia** (cauliflower): soft flowering parts and hard stem part.
2. **Capsicum annum** (chilly):
   - (a) Green chilly: outer cover and seeds.
   - (b) Unripe red chilly: outer cover and seeds.
   - (c) Dried red chilly: outer cover and seeds.
3. **Spices**:
   - (a) *Trigonelia foenumgraecum* (fenugreek): whole grain
   - (b) *Brassica juncia* (mustard seeds): whole grain.
   - (c) *Coriendrum sativum* (coriander): powdery form
   - (d) *Capsicum annum* (red chilly powder):
     - i) Home made.
     - ii) Commercially prepared.
4. **Edibles prepared from paddy/rice**:
   - (a) Boiled flattened paddy (chira).
   - (b) Expanded paddy (khoi).
   - (c) Puffed paddy (muri).
5. **Bread** (locally prepared):
   - (a) Not toasted
   - (b) Toasted.
6. **Cheese**.
7. **Curd**.
8. **Fish**:

   *Cyprinus carpio, Wallago attu, Labeo rohita, Mystus tyngara, Anabas testudineus.*
9. **Meat:**

Boiled in plain water for 30 minutes.

(a) Cold preservation of meat:

i) Goat muscle and liver (24, 48, 72, 96 and 168 hrs. refrigeration).

ii) Chicken muscle and liver (24, 48, 72, 96 and 168 hrs. refrigeration).

iii) Porcine muscle and liver:
- 24, 48, 72, 96 and 168 hour’s refrigeration.
- 30 minutes boiling after 24, 48, 72, 96 and 168 hour’s refrigeration.

iv) Bovine muscle ((24, 48, 72, 96 and 168 hrs. refrigeration).

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**Method**

**Collection and preparation of samples:**

Different parts of *Brassica capitala* (cabbage), *Brassica oleracia* (cauliflower) and *Capsicum annum* (chilly) were collected from local markets through visual selection of fresh looking specimens.

Among the spices, *Trigonelia foenumgraecum* (fenugreek) and *Brassica juncia* (mustard seeds) were used as whole grain. *Coriendrum sativum* (coriander) and *Capsicum annum* (chilly) powder were compared between domestically processed and commercially marketed by some farms,
Microstructural features of muscles were compared between *Cyprinus carpio*, *Cyprinus carpio*, *Wallago attu*, *Labeo rohita*, *Mystus tyngara* and *Anabas testudineus*. Freezing effect on structural changes of *Wallago attu* has also been observed.

Effect of cold preservation on muscular microstructure was studied on different type of meat. Goat, chicken, bovine and porcine muscles were studied after preservation in kitchen refrigerator for 24, 48, 72, 96 and 168 hours.

The effect of boiling followed by freezing for 24, 48, 72, 96 and 168 hours were also conducted in some cases.

**Scanning Electron Microscopy:**

All the samples except powdery spices and edibles prepared from paddy (boiled flattened paddy, expanded paddy and puffed paddy) were processed by routine scanning electron microscopic preparatory techniques as follows:

**Washing:** The samples were cut into small pieces (2mm x 2mm) and then washed thoroughly with double distilled water to remove any extraneous material.

**Fixation:** The emersion fixation procedure was followed for all the samples. The samples were fixed in 2.5-3% glutaraldehyde prepared in 0.1M sodium cacodylate buffer (pH 7.2) for 4 hours at 4°C. Following the primary fixation, the samples were washed in 15-30 minutes in 0.1M sodium cacodylate buffer. The post fixation was carried out with 1% osmium tetroxide prepared in 0.1M sodium cacodylate buffer (pH 7.2).

**Dehydration:** The samples were dehydrated in graded series of acetone (30%, 50%, 70%, 80%, 90%, 95% and dry acetone). The dry acetone was prepared by adding excess
amount of copper sulfate to absolute acetone followed by filtration. The samples were dehydrated in graded acetone with two changes of 15-30 minutes in each grade.

**Drying:** The dehydrated samples were dried either in Critical Point Dryer (SAMDRI-PVT-3, Tousimis) using acetone as intermediate fluid and CO\(_2\) as the transitional fluid or by TMS drying technique of Dey *et al.*, (1989). In the TMS technique, the dehydrated samples were dipped in tetramethylsilane (TMS) at 4°C for 10 minutes. TMS was then changed by fresh TMS and the samples were kept in fresh TMS for another 10 minutes at 4°C. The samples from TMS were placed in glass slide and dried at room temperature (26°C).

**Securing the samples:** The dry samples were secured horizontally to brass stubs (10mm diameter x 30mm height) with double coated adhesive tape, via a patch of silver paint to ensure charged condition.

**Coating:** A conductive coating was applied to the sample using JFC 1100 (Jeol) Ion Sputter Coater. A relatively low vacuum (10\(^{-3}\) torr) was established in the sputtering chamber, and gold was used as the target material.

**Viewing:** The preparations were examined with the Scanning Electron Microscope JSM-6360 (Jeol) using the secondary electron emission mode at an accelerating voltage of 15-20 Kv. Tilt control was fixed at 0° for setting the specimen stage in a horizontal position. Working distance (WD) selector was turned to set the working distance at 38mm.
Results

Scanning electron micrograph of *Brassica oleracia* (cauliflower) flowering part (Fig. 1.1 & Fig. 1.2) showed its highly folded nature, whereas the transverse section of stem (Fig. 1.3) demonstrated the characteristic fibrous nature. Dry red *Capsicum annum* (dry red chilly) skin (Fig. 1.4) and seed cover (Fig. 1.5) demonstrated their characteristic tough nature with grooves at places. But in case of the skin of unripe *Capsicum annum* (unripe red chilly) deep folding were observed (Fig. 1.6). SEM of *Daucus corota* (carrot, Fig. 1.7) and *Dolichos lablab* (flat bean, Fig. 1.8) represented normal cellular morphology of xylem under SEM which is known to differ as functions of maturity, infection, quality etc. *Coriendrum sativum* (coriander) leaf surface (Fig. 1.9 & 1.10) showed highly folded nature with large distribution of stomata. Outer surface of *Phaseolus vulgaris* (french bean) when observed under SEM (Fig. 1.11) detailed highly folded nature. Sectional view on the other hand (Fig. 1.12) and also the seed of the same vegetable (Fig. 1.13 & 1.14) indicated the occurrence of protein bodies of different shapes and sizes. The seed cover of this vegetable (Fig. 1.13) was also found to be folded. *Pisum sativum* (garden pea) (Fig. 1.15) was found to contain protein bodies as well. *Abelmoscus esculentus* (ladies finger) cross section revealed (Fig. 1.16) the presence of fat bodies with different shapes and sizes. However, the outer surface of the same vegetable (Fig. 1.17) showed folded nature along with some fibrous outgrowth. Cross section of *Solanum tuberosum* (potato) (Fig. 1.18) showed a large number of starch bodies of different sizes. The outer surface of *Trichosenthis dioica* (pointed gourd) (Fig. 1.19) was also found to be folded with thick outer boundary surrounding inner pits. Protein bodies were evident in this species under cross sectional view (Fig. 1.20). The outer surface of *Lycopersicum esculentum* (tomato, Fig. 1.21) was found to be more or less
smooth with the presence of some pits or grooves throughout. The sectional view on the other hand (Fig. 1.22), showed the presence of somewhat crystalline structure. Surface microstructure of *Trachyspermum ammi* (ajwan) showed abundance of stomata with dense concentration of cotyledon (Fig. 1.23). *Cajanus cajan* (arhar dal) and *Lens culinaris* (musur dal) surface microstructure reveals dense conglomeration of protein bodies, characteristic grooves and pit like structures (Fig. 1.24 & 1.25). On the other hand, surface microstructure of *Nigella sativa* (cumin black) showed thick aggregation of scale like structures (Fig. 1.26). Cross section of *Brassica juncea* (mustard) seeds showed fat, as well as oil bodies of different shapes and sizes (Fig. 1.27). Microstructure of the surface of *Phaseolus radiatus* (kala dal) revealed tough outer covering with dense aggregation of grooves and pits (Fig. 1.28).

Different rice products which have the same nutrient content showed differences in their microstructure. In case of boiled rice (*Oriza sativa*) the sectional view of a single grain showed tough linear nature of the constituents, mainly the carbohydrate (Fig. 1.29). Boiled flattened rice (chira) also showed the similar toughness and the starch bodies of various shapes and sizes were observed (Fig. 1.30). In case of popped boiled rice (khoi) (Fig. 1.31) large air spaces indicate its lightness and relatively less amount of the major carbohydrate content as compared to boiled rice and boiled flattened rice (chira). In case of puffed boiled rice (muri) (Fig. 1.32) the condition is somewhat similar to that of popped boiled rice (khoi) but to a lesser extent as far as the air space is concerned.

The SEM of locally prepared curd showed the presence of fat globules mixed with protein bodies (Fig. 1.33). The scanning electron micrograph of locally prepared bread in un-toasted condition showed the large size of starch bodies (Fig. 1.34) with thick fibrils of

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gluten. However in the toasted one, shrinkage of the starch bodies as well as gluten is evident (Fig. 1.35).

Microstructure of different home made spices such as Capsicum annum (chilly) powder (Fig. 1.36), Coriendrum sativum (coriander) powder (Fig. 1.37), Cuminum cyminum (cumin) powder (Fig. 1.39 & 1.40) etc. showed similar features in terms of arrangement and size of protein bodies. Commercially available Coriendrum sativum powder (coriander powder) sometimes shows some foreign bodies, e.g., rodent hair (Fig. 1.38). Trigonella foenum graecum (fenugreek) seed coat and cotyledon and Brassica juncia (mustard) seed coat (Fig. 1.41 & 1.42) and cotyledon also showed similar features in terms of arrangement and distribution of cotyledon. However, Brassica juncia (mustard) seed coat (Fig. 1.43) showed some toughness as revealed from thick boundary between the adjacent plates. The micrograph of home made Curcuma longa (turmeric) powder revealed the clusters of starch particles (Fig. 1.44).

Microstructure of wallago attu muscle fibres in fresh condition revealed the presence of normal compactness of the musculature (Fig. 1.45) with uniform ridges formed from transverse elements of sercoplastic reticulum (Fig. 1.46). But the same musculature after 24h freezing at 4°C showed the beginning of toughness in the muscle fibres (Fig. 1.47) in the form of high compactness. The same muscle fibres after 48h of freezing (Fig. 1.48), 72h freezing (Fig. 1.49) and 96h freezing (Fig. 1.49) at 4°C demonstrated gradual increase in toughness through compact arrangement of muscle fibres. After 168h of freezing at 4°C the muscle fibres of Wallago attu showed very high degree of compactness of fibres (Fig. 1.51). The muscle fibres of Labeo rohita in fresh condition showed relaxed sercomeres and myofibrils (Fig. 1.52 & 1.53). But after 36hrs of freezing at 4°C the same muscle fibres
started showing the characteristic toughness (Fig. 1.54 & 1.55) through compactness of fibres. In case of *Mystus tyngara* muscle fibres in fresh condition showed smooth contour of the fibres (Fig. 1.56), while the roughness was observed in *Anabas testudineus* in fresh condition (Fig. 1.57) as compared to that of *Mystus tyngara*.

Chicken muscle in fresh condition showed the normal compactness of muscle fibres with normal perimysium (Fig. 1.58). But after 48h of freezing at 4°C the muscle is found to take tougher appearance (Fig. 1.59). Cross section of chicken liver shows normal distribution of fat globules and other components (Fig. 1.60), but gradual abnormality in size and shape of fat bodies were observed when kept in freezer at 4°C for 24h and 48h (Figs. 1.61 & 1.62). Under fresh condition, goat muscle showed the normal compactness of the fibres along with perimysium (Fig. 1.63), while boiled in water for 30min, these muscle fibres were found to be loosened (Fig. 1.64). In contrast, when the goat muscle was kept in freezer at 4°C for 24h the greater compactness and toughness appeared (Fig. 1.65). When this muscle fibres (kept in freezer for 24h at 4°C) were boiled in water for 30min, the loosening and breaking of muscle fibres to some extent was observed (Fig. 1.66). When kept at 4°C for 48h the muscle fibres have been found to be more compact (Fig. 1.67) while duration was prolonged to 72h at the same temperature, rigidity of the fibres become obvious (Fig. 1.68). Negligible change of the muscle compactness, as revealed from microstructure, has been observed when the same muscle (frozen for 72h) was boiled in water for 30min (Fig. 1.69). The muscle fibres of goat seem to toughen further when kept in freeze at 4°C for 96h (Fig. 1.70), but showed little or no change in microstructure when boiled in water for 30min (Fig. 1.71). The observation of microstructure of goat muscle fibres, kept in the freezer at 4°C for 168h showed obvious toughness (Fig. 1.72) and 30min
boiling of the same muscle fibres presented little effect on the fibres (Fig. 1.73). The microstructure of goat liver in fresh condition showed normal features (Fig. 1.74) such as relaxed musculature, fat bodies etc. However, boiling for 30min could breakdown the muscles and the fat bodies (Fig. 1.75). When the goat liver is kept in freeze at 4°C for 24h fat and oil bodies could be observed (Fig. 1.76). After 48h of freezing at 4°C the goat liver microstructure did not show much of differences (Fig. 1.77) from that with 24h of freezing. However, boiling for 30min of the same seems to have little effect on the liver microstructure (Fig. 1.78). After 72h of freezing at 4°C, the liver muscles along with fat and oil globules seem to take crystalline shape (Fig. 1.79), with little or no effect after 30min boiling as revealed from microstructural features (Fig. 1.80). The rigidity and compactness of the liver tissues become evident when kept at 4°C for 96h (Fig. 1.81). The compactness and toughness becomes more evident when the goat liver is kept at 4°C for 168h (Fig. 1.82).

Porcine muscle at fresh condition showed smooth muscle fibres with parts of connective tissues attached to it (Fig. 1.83), and when the same is boiled for 30min the softening and breaking of the fibres became evident (Fig. 1.84). After 36h of freezing at 4°C the same muscle fibres started to show tough and rigid characters (Fig. 1.85). The porcine muscle showed gradual increase of toughness and rigidity with subsequent increase in time of exposure to freezing i.e., 72h (Fig. 1.86), 96h (Fig. 1.87) and 168h (1.88). Toughness of muscle fibres remained the same when the muscle (after 168h freezing at 4°C) had been boiled in water for 30min (Fig. 1.89). The microstructural features of fresh porcine liver showed arrangement of fine fibres (Fig. 1.90). Freezing for 24hrs at 4°C revealed some toughness and crystallinity of the same (Fig. 1.91). After 72h of freezing at 4°C the microstructure of porcine liver showed the clumping of fat bodies (Fig. 1.92). After 168h of
freezing at 4°C the degree of clumping had been found to increase (Fig. 1.93). Bovine muscle at fresh condition showed normal relaxed condition of the fibres with relatively smooth contour (Fig. 1.94). After 30min boiling in water the same showed softening and breaking (Fig. 1.95). After 24h of freezing at 4°C the microstructure of bovine muscle fibres started to show tough configuration (Fig. 1.96). This toughness had been found to increase gradually with increase of freezing time i.e., at 36h freezing (Fig. 1.97), 72h of freezing (Fig. 1.98) and 96h of freezing (Fig. 1.99). Almost no microstructural change had been observed when the muscle with 96h freezing had been boiled for 30min (Fig. 1.100). Rigidity and toughness of the bovine muscle were quite evident after 168h of freezing at 4°C (Fig. 1.101) and almost no microstructural change had been observed of the same when boiled for 30min (Fig. 1.102).
Fig. 1.1. *Brassica oleracea* (cauliflower) flowery part. Bar = 100μm (X150).

Fig. 1.2. *Brassica oleracea* (cauliflower) flowery part showing folding (F). Bar = 20μm (X700).

Fig. 1.3. *Brassica oleracea* (cauliflower) stem showing roughage. Bar = 20μm (X700).

Fig. 1.4. Dry red *Capsicum annum* (red chilly) skin. Bar = 10μm (X1100).

Fig. 1.5. Dry red *Capsicum annum* (red chilly) seed cover with groves (G). Bar = 500μm (X33).

Fig. 1.6. Red unripe *Capsicum annum* (unripe red chilly) skin showing deep folding (F). Bar = 20μm (X550).
Fig. 1.7. *Daucus corota* (carrot) showing pattern of xylem. Bar = 50μm (X500)

Fig. 1.8. *Dolichos lablab* (flat bean) showing pattern of xylem. Bar = 100μm (X250)

Fig. 1.9. *Coriendrum sativum* (coriander) leaf surface showing considerably large distribution of stomata (arrows). Bar = 50μm (X500)

Fig. 1.10. Enlarged view of *Coriendrum sativum* (coriander) leaf surface showing primary (PF) and secondary (SF) folding. (S=stomata). Bar = 20μm (X950).

Fig. 1.11. *Phaseolus vulgaris* (French bean) outer surface. Bar = 10μm (X1500)

Fig. 1.12. *Phaseolus vulgaris* (French bean) T/S showing protein bodies (P) of various sizes. Bar = 10μm (X2500)
Fig. 1.13. *Phaseolus vulgaris* (French bean) seed cover outer side showing number of cracks. Bar = 10μm (X1500)

Fig. 1.14. *Phaseolus vulgaris* (French bean) seed T/S showing protein bodies (P). Bar = 10μm (X1000)

Fig. 1.15. *Pisum sativum* (garden pea) with protein granules (P). Bar = 10μm (X2500)

Fig. 1.16. *Abelmoschus esculentus* (ladies finger) T/S showing granular fat bodies (F). Bar = 5μm (X3000)

Fig. 1.17. *Abelmoschus esculentus* (ladies finger) outer surface showing highly folded nature (F) with fibrous outgrowth (FO). Bar = 20μm (X550)

Fig. 1.18. *Solanum tuberosum* (potato) T/S showing starch granules (S) of various sizes. Bar = 20μm (X600)
Fig. 1.19. *Trichosenthes dioica* (pointed gourd) outer surface showing mosaic of thick folding (F). Bar = 10μm (X1000)

Fig. 1.20. *Trichosenthes dioica* (pointed gourd) T/S showing protein bodies (P). Bar = 10μm (X2500)

Fig. 1.21. *Lycopersicum esculentum* (tomato) outer surface showing smoothness with grooves (G). Bar = 20μm (X700)

Fig. 1.22. *Lycopersicum esculentum* (tomato) T/S showing crystalline inner structure (C). Bar = 20μm (X850)

Fig. 1.23. *Trachyspermum ammi* (ajwan) surface showing stomata (S) and cotyledon (C). Bar = 20μm (X600)

Fig. 1.24. *Cajanus cajan* (arhar-dal) surface showing rosette like structure. Bar = 50μm (X500)
Fig. 1.25. *Lens culinaris* (musur-dal) surface showing even distribution of pits (PP). Bar = 5μm (X4000)

Fig. 1.26. *Nigella sativa* (cumin black) surface showing scale like structures. Bar = 100μm (X250)

Fig. 1.27. Cross section of *Brassica juncea* (mustard) seed showing normal distribution of fat (F) and protein (P) bodies. Bar = 20μm (X650)

Fig. 1.28. *Phaseolus radiatus* (kala-dal) showing undulating pits and grooves. Bar = 20μm (X850)

Fig. 1.29. Boiled rice (*Oryza sativa*) cut part showing relatively tough nature of the constituents, mainly starch. Bar = 20μm (X550)

Fig. 1.30. Boiled flattened rice (Chira) T/S showing relatively tough nature of the constituents. Starch particles (S) of various sizes are seen. Bar = 5μm (X3000)
Fig. 1.31. Popped boiled rice (Khoi) showing large air spaces (AS). Bar = 50μm (X500)

Fig. 1.32. Puffed boiled rice (Muri) showing less air spaces as compared to popped boiled rice (Khoi). Bar = 10μm (X1000)

Fig. 1.33. Locally prepared curd showing fat globules (F) mixed with protein bodies (P). Bar = 5μm (X5000)

Fig. 1.34. Section of locally prepared bread un-toasted showing large starch granules (S) and thick fibrils of gluten (F). Bar = 10μm (X2000)

Fig. 1.35. Section of locally prepared bread (toasted) showing smaller starch granules (S), thinner fibrils of gluten (F) and protein bodies (P). Bar = 10μm (X2000)

Fig. 1.36. Home made chilly powder showing normal pattern of protein bodies. Bar = 10μm (X3000)
Fig. 1.37. Home made *Coriandrum sativum* (coriander) powder showing normal pattern of protein bodies. Bar = 10\(\mu\)m (X860)

Fig. 1.38. Commercially available *Coriandrum sativum* (coriander) powder showing foreign body, a rodent hair. Bar = 10\(\mu\)m (X780)

Fig. 1.39. Home made *Cuminum cyminum* (cumin) powder showing normal pattern of protein bodies. Bar = 10\(\mu\)m (X1100)

Fig. 1.40. Home made *Cuminum cyminum* (cumin) powder showing distribution of protein bodies. Bar = 100\(\mu\)m (X320)

Fig. 1.41. *Trigonella foenumgraecum* (fenugreek) cotyledon showing uniformity. Bar = 10\(\mu\)m (X1200)

Fig. 1.42. *Brassica juncea* (mustard) seed cotyledon showing uniformity in distribution and size. Bar = 10\(\mu\)m (X1200)
Fig. 1.43. *Brassica juncea* (mustard) seed coat showing tough outer covering. Bar = 10μm (X600).

Fig. 1.44. Home made *Curcuma longa* (turmeric) powder showing starch particle cluster. Bar = 10μm (X1100).

Fig. 1.45. *Wallago attu* control muscle fibres showing normal compactness of musculature. (MS = muscle fibres). Bar = 100μm (X190).

Fig. 1.46. *Wallago attu* control muscle fibres showing uniform ridges formed from transverse elements of sercoplasmic reticulum (SR). Bar = 5μm (X4000).

Fig. 1.47. *Wallago attu* muscle fibres after 24h freezing at 4°C. (MS = muscle fibres). Bar = 5μm (X4500).

Fig. 1.48. *Wallago attu* muscle fibres after 48h freezing at 4°C showing increased compactness of the muscle fibres. Bar = 5μm (X3300).
Fig. 1.49. *Wallago attu* muscle fibres after 72h freezing at 4°C, showing high degree of compactness of the muscle fibres. (MF= muscle fibre). Bar = 50μm (X350)

Fig. 1.50. *Wallago attu* muscle fibres after 96h freezing at 4°C with more prominence of compactness. (MF= muscle fibre). Bar = 10μm (X2200)

Fig. 1.51. *Wallago attu* muscle fibres after 168h freezing at 4°C showing a very high degree of compactness (MF= muscle fibre). Bar = 10μm (X2500)

Fig. 1.52. *Labeo rohita* muscle fibres in fresh condition showing relaxed condition of the sarcomares (S) and myofibrils (M). Bar = 100μm (X220)

Fig. 1.53. *Labeo rohita* muscle myofibril in fresh condition at higher magnification. Bar = 5μm (X4000)

Fig. 1.54. *Labeo rohita* muscle fibres after 36h freezing at 4°C showing relatively high degree of compactness of the fibres. (MF=muscle fibre). Bar = 20μm (X950)
Fig. 1.55. *Labeo rohita* muscle fibres after 36h freezing at 4°C at higher magnification. Toughness of the fibres is evident. (MF=muscle fibre). Bar = 5μm (X3300)

Fig. 1.57. *Anabas testudineus* muscle fibres control showing roughness in comparison to that of *Mystus tyngara*. (MF=muscle fibre). Bar = 50μm (X500)

Fig. 1.59. Chicken muscle fibres (MF) after 48h freezing at 4°C showing crystalline perimysium (P). Bar = 100μm (X250)

Fig. 1.56. *Mystus tyngara* muscle fibres control, showing smooth contour of the fibres. (MF=muscle fibre). Bar = 50μm (X370)

Fig. 1.58. Chicken muscle fibres (MF) in fresh condition with normal perimysium (P). Bar = 50μm (X400)

Fig. 1.60. Cross section of chicken liver control showing distribution of fat globules (F). Bar = 10μm (X1200)
Fig. 1.61. Cross section of chicken liver after 24h freezing at 4°C. Bar = 10μm (X1200)

Fig. 1.62. Cross section of chicken liver after 48h freezing at 4°C showing constricted fat bodies (F). Bar = 10μm (X1200)

Fig. 1.63. Goat muscle fibres (fresh) showing normal compactness of muscle fibres (MF) and perimysium (P). Bar = 20μm (X600)

Fig. 1.64. Goat muscle fibres (fresh) after 30min boiling. Compact muscle fibres (MF) are loosened. Bar = 20μm (X600)

Fig. 1.65. Goat muscle fibres after 24h freezing at 4°C showing the beginning of toughness of the fibres (MF). Bar = 50μm (X300)

Fig. 1.66. Goat muscle fibres (MF), 30min boiling after 24h freezing at 4°C. Bar = 20μm (X600)
Fig. 1.67. Goat muscle fibres (MF) after 48h freezing at 4°C showing compactness of muscle fibre. Bar = 20μm (X550)

Fig. 1.68. Goat muscle fibres (MF) after 72h freezing at 4°C showing greater degree of compactness. Bar = 10μm (X1200)

Fig. 1.69. Goat muscle fibres (MF) 30min boiling after 72h freezing at 4°C showing negligible change in the toughness of the muscle fibres. Bar = 10μm (X1000)

Fig. 1.70. Goat muscle fibres (MF) after 96h freezing at 4°C. Toughness of the muscle fibres are evident. Bar = 10μm (X1000)

Fig. 1.71. Goat muscle fibres (MF), 30min boiling after 96h freezing at 4°C. Not much change in the toughness of the fibres. Bar = 10μm (X2000)

Fig. 1.72. Goat muscle fibres (MF) after 168h freezing at 4°C. Bar = 10μm (X1000).
Fig. 1.73. Goat muscle fibres (MF), 30min boiling after 168h freezing at 4°C. No apparent affect of boiling on the toughness. Bar = 10μm (X1000)

Fig. 1.74. Cross section of goat liver (fresh). Bar = 20μm (X550)

Fig. 1.75. Cross section of goat liver (fresh), after 30min boiling. (F = fat bodies). Bar = 10μm (X2200)

Fig. 1.76. Cross section of goat liver after 24h freezing at 4°C. Fat and oil bodies can be observed. Bar = 50μm (X500)

Fig. 1.77. Cross section of goat liver after 48h freezing at 4°C. Bar = 50μm (X500)

Fig. 1.78. Cross section of goat liver with 30min boiling after 48h freezing at 4°C. Not much change in the texture can be observed. Bar = 50μm (X500)
Fig. 1.79. Cross section of goat liver after 72h freezing at 4°C. The fat and oil globules are taking crystalline state. Bar = 10μm (X2500)

Fig. 1.80. Cross section of goat liver with 30min boiling after 72h freezing at 4°C. Not much structural change is observed. Bar = 10μm (X1000)

Fig. 1.81. Cross section of goat liver, after 96h freezing at 4°C. Rigidity of different liver components is evident. Bar = 10μm (X1000)

Fig. 1.82. Cross section of goat liver, after 168h freezing at 4°C. Bar = 5μm (X4000)

Fig. 1.83. Fresh porcine muscle. Smooth muscle fibres (MF) are seen. (CT=parts of connective tissue). Bar = 10μm (X600)

Fig. 1.84. Fresh porcine muscle after 30min boiling. Softening of muscle fibres (MF) is obvious. Bar = 10μm (X600)
Fig. 1.85. Porcine muscle after 36h freezing at 4°C. Muscle fibres (MF) are beginning to look more compact. Bar = 10μm (X600)

Fig. 1.86. Porcine muscle after 72h freezing at 4°C. (MF= muscle fibres). Bar = 10μm (X600)

Fig. 1.87. Porcine muscle after 96h freezing at 4°C. (MF= muscle fibres). Bar = 10μm (X1200)

Fig. 1.88. Porcine muscle after 168h freezing at 4°C. (MF= muscle fibres). Bar = 100μm (X300)

Fig. 1.89. Porcine muscle with 30min boiling after 168h freezing at 4°C. Toughness of the muscle fibres (MF) is still evident. Bar = 10μm (X1200)

Fig. 1.90. Cross section of porcine liver (fresh). Bar = 10μm (X2300)
Fig. 1.91. Cross section of porcine liver after 24h freezing at 4°C. Bar = 50μm (X300)

Fig. 1.92. Cross section of porcine liver after 72h freezing at 4°C. Fat bodies (FB) are clumping together. Bar = 10μm (X1100)

Fig. 1.93. Cross section of porcine liver after 168h freezing at 4°C. Clumping of fat bodies (FB) are evident. Bar = 10μm (X1500)

Fig. 1.94. Fresh bovine muscle control (MF= muscle fibres). Bar = 5μm (X4000)

Fig. 1.95. Fresh bovine muscle control after 30min boiling. Softening and breaking of muscle fibres (MF) is observed. Bar = 20μm (X900)

Fig. 1.96. Bovine muscle after 24h freezing at 4°C. (MF= muscle fibres). Bar = 20μm (X900)
Fig. 1.97. Bovine muscle after 36h freezing at 4°C. (MF= muscle fibres). Bar = 100μm (X200)

Fig. 1.98. Bovine muscle fibres (MF) after 72h freezing at 4°C. Toughness of muscle fibres is evident. Bar = 100μm (X220)

Fig. 1.99. Bovine muscle after 96h freezing at 4°C. (MF= muscle fibres). Bar = 100μm (X200)

Fig. 1.100. Bovine muscle with 30min boiling after 96h freezing at 4°C. Almost no structural change is observed in the muscle fibre. Bar = 20μm (X900)

Fig. 1.101. Bovine muscle after 168h freezing at 4°C. Toughness of muscle fibres (MF) is obvious. Bar = 100μm (X200)

Fig. 1.102. Bovine muscle with 30min boiling after 168h freezing at 4°C. Rigidity and toughness of the muscle fibres (MF) are evident. Bar = 20μm (X800)
Discussion

Microstructure gives the food its characteristic properties. Microscopy provides the specific feature for describing how a particular structure is engineered and how it relates to the properties. This allows us to study the effect of a change in one bio-polymer within a complex assembly and to follow how such a change can affect the behaviour of the overall structure (Hermansson et al., 2000). This provides a clue for exploring the bio-physical reasons behind the relationship between structures and behaviors such as mechanical properties.

On this consideration, microstructural studies on different edibles carried out here appears to be relevant (Tangwongchai et al., 2000; Mohamed et al., 2002; Bai et al., 2002; Murata et al., 2002). The observations made in the present study on the microstructural characteristics of different vegetables is thus expected to serve as a spring-board for future studies regarding changes associated with environmental stress and pathological conditions. The structural features of the Coriendrum sativum (coriander) leaves, Brassica oleracia (cauliflower) etc, suggest that abundance of stomata and highly folded nature of the surface increases its capacity for trapping and conserving solar energy which consequently will determine the nutrient quality. Any deviation from this natural microstructural features due to pathogenicity or environmental stress may be used to determine the nutrient quality based on the present configuration. Similarly, the ultrastructural features of xylem in Dancus corota (carrot) and Dolichos lab lab (flat bean) presented here may serve as a reference for future study in relation to abnormalities, if any, since it is known that these features are related to the quality and variety of the concerned vegetable (Davis & Gordon 1980).
The folded nature of *Phaseolus vulgaris* (french bean) surface and the distribution pattern of protein bodies in sectional view; protein bodies in *Pisum sativum* (garden pea); distribution pattern of fat bodies in the cross sectional view of *Abelmoscus esculentus* (ladies finger); folding and fibrous outgrowth on *Abelmoscus esculentus* (ladies finger) surface; starch bodies in *Solanum tuberosum* (potato); thick folding and pits on the surface of *Trichosenthis dioica* (pointed gourd); size and distribution pattern of protein bodies in the cross section of *Trichosenthis dioica* (pointed gourd); grooves on the outer surface of *Lycopersicum esculentum* (tomato) along with general smoothness of the surface and the crystalline structures appeared in section of *Lycopersicum esculentum* (tomato) presented here are the normal microstructural features of the afore mentioned vegetables. The relevance of this study lies in the fact that, normal microstructural features of these can serve as a reference in determining the quality because any abnormality due to the environmental stress or pathogenic conditions is likely to be expressed in it.

Many food processing operations are designed to create the microstructure that gives the food product its characteristic properties. In this context, the present study concerning the microstructural features of different rice products appears to be relevant. Tough linear nature of the structural components in rice and boiled flat-rice (Chira) suggests the specific suitability of these products. It appears that for infant, sick and elderly people with poor digestive ability, these products could be consumed after proper preparations and processing. The air space found in boiled popped rice (Khoi) and boiled puffed rice (Muri) indicates that these may be easily digestible because of their lightness, and relatively low carbohydrate contents and as compared to similar quantity of rice or
boiled flattened rice (Chira) and may be used by persons with specific ailments (e.g., diabetes).

Fat globules mixed with protein in locally prepared curd indicate that there are no abnormal features as far as the microstructure is concerned. The microstructure of curd and other dairy products have been studied by different authors who described the similar features presented in this study (Glaser et. al., 1979; Glaser et. al., 1980; Slattery 1976; Dabour et. al., 2006). Any kind of defects in the preparation are likely to be reflected in its microstructure. Hence, the present study can act as a reference for future investigations on dairy products as well. The larger starch bodies and thicker gluten fibres in un-toasted locally prepared bread and shrinkage of the same in toasted ones may also be related to its digestibility.

The surface feature of Capsicum annum (red chilly) powder, Coriendrum sativum (coriander) powder, Brassica juncea (mustard) seed coat, Trigonella foenum-graecum (fenugreek) cotyledon, Curcuma longa (turmeric) powder etc. revealed smoothness indicating that difficulties associated with digestion due to consumption of spices may not be related to their structural features. However, presence of foreign bodies and some coarse particles in some commercially prepared spices may cause various types of problems. The absence of unwanted foreign bodies in home made spice powder suggests that consumption of commercially prepared spice-powders should be made with appropriate precautions.

Among the different aspects of food microstructures the most extensively addressed area appears to be the microstructure of muscles of different animal. Basrur et. al., (1983) studied the myofibrillar characteristics of porcine stress syndrome. Cassens
et.al., (1984) made an analysis of microstructural factors which influence the use of muscle as food. Lee (1985) studied the microstructure of meat emulsion in relation to fat stabilization. However, despite the fact that voluminous works on muscle microstructure and its changes due to processing in bovine and porcine muscle were done, but very little work has been carried out in this regard on fish muscles. The present observations on differences in toughness and smoothness of surface features of muscles in a number of fish species indicates that species to species variations may exist in fish as far as their muscle microstructure is concerned. Consequently, types of fish may have to be selected on the basis of digestive capability of a consumer such as sick individuals, children and elderly people.

As far as the preservation of fish and meat is concerned, the present study suggests that it is desirable to consume fish and meat in their fresh state. However, in unavoidable circumstances they may be preserved through refrigeration but, not for a period more the 24 to 36 hours. This is because of the fact that the present study revealed considerably high degree of muscle toughness after freezing for more than 36 hours. In that case, boiling for even a long period of time cannot break or smooth the muscle bundles to be digested easily. In this regard it may not be illogical to state that, deep frozen broiler chicken available in the markets and consumed by many is not as good as fresh chicken as far as its muscle toughness and food quality is concerned.

Electron microscopic approaches on food microstructure appears to be a promising field in determining the wholesomeness of food and its quality, combined with biochemical and bioinorganic studies. It is surprising to note that despite the understanding of wide applicability and importance of food microstructure, the study is
restricted to some western countries (Berrios et.al., 1998; Garcia et.al., 1999; Yamada et.al., 2000; Foster et.al., 2002; Kalab 2000). In context of India in general and Northeast India in particular the present study appears to be the first of its kind as far as the food microstructure is concerned.

Although the study is of general and preliminary in nature, it is expected that this will serve as a platform for further studies. Specific studies on different components on various types of food materials are likely to open up a new area of research on food quality. The observations made here on muscle of fish and other animals in relation to freezing time provides us with the data on optimal time for freezing of these edibles. However, the study did not address to the environmental pollution aspects with reference to food microstructure. It is worthwhile to mention in this context that different structural components of fish have been found to show characteristic microstructural abnormalities in response to specific pollutants (Dey et.al., 2001). Similar types of studies may be important in different edibles to know the microstructural deterioration caused by pollutants which will determine the acceptability or rejection of the food with reference to human health.
Summary:

- Detection of folded nature and fibrous structure in some edibles through scanning electron microscopy.

- Demonstration of normal cellular morphology of xylem in some plant edibles, which is known to be characteristics in terms of maturity, infection, quality etc.

- Demonstration of protein bodies of different sizes and shapes in some edibles of plant origin.

- Detection of fat bodies in some edibles of plant origin, such as *Brassica juncea* (mustard) seeds etc.

- Detection of differential microstructural features revealing smoothness or toughness of muscles in different fish species.

- Observations on rigidity or toughness of muscle in different types of meat in long-freezing treatment,