Characterization of food texture: application of Microscopic technology

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This review is a survey on the latest and more recent applications of microscopy to study the morphological changes that food components or microorganisms undergo during processing. Apart from a brief discussion on the fundamentals of microscopic techniques, examples have been set out on the physical effects of different processes such as drying (hot air drying, spray drying, microwave, osmotic drying, freeze drying and superheated steam drying), freezing, high hydrostatic pressure, pulsed electric fields, and ultrasound on the microstructure and three-dimensional surface morphology of a wide range of different food materials. Selected examples of microscopic studies of food structure are presented to illustrate the potential of this technique to reveal detailed and specific information. Understanding the relation between processing conditions and food structure, by the use of microscopy, can be helpful for improving food processes or defining the proper conditions that help to retain the quality of the product.

Keywords microscopy technique; scanning electron microscopy; microstructure; food processing

1. Introduction

Microscopy is being increasingly used to study the influence of processing conditions and ingredients on food structure and only in the last few decades the full potential of electron microscopy (EM) has been recognized. [1] Recent developments in these fields have changed our understanding not only about food structures but also about the types of information which can be now expected to glean regarding structure. Three dimensional imaging, minimal sample intervention and in situ microscopy for dynamic studies, coupled with a greater appreciation of the power of image analysis to derive quantitative information from microscopic images are becoming more common. [2, 3] Scanning electron microscopy (SEM) is a very useful tool to visualize food structure because; it combines in many ways the best features of light microscopy (LM) and transmission electron microscopy (TEM). [4] Several excellent reviews of applications of SEM to food studies exist, including books by Holcomb and Kalab, [5] and a section of Aguilera and Stanley [6]. In addition to these, papers summarizing the applications of SEM [6, 7] and cryo-SEM [8] provide a valuable introduction to the aspects of food structure that can be revealed by these techniques.

The history of electron microscopy began with the development of electron optics. In 1926, Busch studied the trajectories of charged particles in axially symmetric electric and magnetic fields, and laying the foundations of geometrical electron optics showed that such fields could act as particle lenses. [9] Nearly at the same time, French physicist de Broglie introduced the concept of corpuscle waves. A frequency and hence a wavelength was associated with charged particles: wave electron optics began. Following these two discoveries in electron optics, the idea of an electron microscope began to take shape. The first true SEM was described and developed in 1942 by Zworykin, who showed that secondary electrons provided topographic contrast by biasing the collector positively relative to the specimen. [10] Compared to light microscopy, SEM has been very attractive for food scientists because both surface and internal features can be studied, a wide range of magnification is possible and the SEM can achieve a depth of field roughly 500 times greater than that of light microscopy.

In recent years, the study of the microstructure of food has taken on increasing significance since; the structure of foods can have a profound influence on its nutritional value, rheology and textural attributes. Food processing, such as thermal and nonthermal processes, can be thought of as altering the natural structure and the composition of food materials. [11] However, heat processing, particularly under severe conditions, may give rise to chemical and physical changes that impair the organoleptic properties and reduce the content or bioavailability of some nutrients. Therefore, the food industry is constantly searching for emergent mild processing technologies such as high hydrostatic pressure (HHP), pulsed electric fields (PEF), ultrasound (US), and irradiation, among others, not only to obtain high-quality food with “fresh-like” characteristics, but also food with improved or even novel functionalities. [12] All of these technologies are considered as nonthermal technologies, because the main preservation factor is different from heat (i.e. pressure, electricity, sound waves, light or radiation) and most of them conducted at room temperature. [13]

This review article covers use of microscopy in the study of food structure and highlights the effect of different processes on the microstructure of food ingredients. Different common microscopic techniques are briefly summarized for analysis of food structure. Numerous examples and images are included to demonstrate the richness of information that can be obtained by the discussed techniques. The effects of different processes such as different kinds of drying, freezing, high hydrostatic pressure, pulsed electric fields, and ultrasound on the microstructure of food ingredients or microorganisms have been reported in this study.
2. Electron microscopy

Electron microscopy has been widely employed for the evaluation of the microstructure of food and biological products. In this method, electron beams are used to observe the specimen rather than light and therefore preparation is needed for biological materials. This preparation is much complicated than that required for light microscopy. Different electron microscopy techniques including transmission electron microscopy (TEM), scanning electron microscopy (SEM), cryo-SEM, cryo-TEM, and environmental scanning electron microscopy (ESEM) have been employed for the evaluations related to process of food.

In SEM the image is formed step by step with scanning a focused electron beam across the specimen. The primary electrons penetrate the solid specimen and are deflected by a large number of elastic scattering processes. Various signals are generated as results of the impact of the incident electrons which collected to form an image or to analyse the sample surface. These are mainly secondary electrons, with energies of a few tens of eV, high-energy electrons back-scattered from the primary beam and characteristic X-rays. Many advantages of SEM have been exploited in examining food microstructure. These include relatively simple sample preparation, wide range of magnification, high depth of field and the fact that the image is a representation of electronic data allowing for image analysis and quantification. It has been described as a technique that combines the best aspects of light microscopy and transmission electron microscopy. The disadvantages of conventional SEM techniques still exist, predominantly the difficulties associated with examining insulating specimens and the impossibility of examining hydrated samples without altering their state in some way (either drying or freezing). These sample preparation treatments introduce artifacts and, literally or figuratively, freeze the sample meaning that studies of dynamic processes must be interrupted for examination.

Cryo scanning electron microscopy (cryo-SEM) is a direct method of observing the ice crystals of frozen samples without prior thawing. In this technique additional steps such as coating with metal for enhanced conductance of the electrons are usually performed. The frozen specimens prepared for cryo electron microscopy are not replicated, but are immediately transferred to a low temperature stage within the microscope and viewed directly. The cryo-SEM method provides rapid physical fixation and avoids the risk of introducing artefacts entailed in chemical fixation, structural collapse or shrinkage as sometimes occurs in typical preparation methods, therefore the advantages of this technique include the preservation of triphasic structure of a sample with the distribution of solid, liquid and gas in a state closer to its natural state. However, similar to SEM method, its main drawback is its high cost.

Environmental SEM differs from conventional SEM mainly by the presence of a gas in the specimen chamber. Samples are thus not viewed under high vacuum but under a deteriorated or "low" vacuum. This is possible thanks to a special design of electron optics column that allows differential pumping: the column is divided into different pressure zones separated by pressure limiting apertures. The presence of gas, or environment around the sample that inspired the term "environmental" SEM, can play two main roles. The first, common for environmental as well as low-vacuum SEMs, is electronic. The gas acts as an electrical charge conductor avoiding sample charging and facilitates signal detection. The second role, more specific to environmental SEMs, is thermodynamic, i.e. the gas is a conditioning medium, preventing evaporation of liquids from a sample.

Conventional transmission electron microscopes (TEM) are electron optical instruments analogous to light microscopes. However, the specimen is not illuminated by light, but by an electron beam. Since gas molecules would deflect the electrons, this requires operation in a vacuum. An electron gun is at the top of the microscope column and a system of electromagnetic lenses focuses the electron beam on the sample. The image contrast in TEM is obtained by the interactions of electrons with the material, i.e. electron scattering. The resolution in TEM is directly proportional to the acceleration voltage of the electrons. High resolution is obtained due to the short wavelength of the electrons when the voltage increases. However, increasing acceleration voltage leads to poorer contrast since scattering of electrons is decreased in the higher velocity. Typical instruments are capable of voltages from 40 to 120 kV and microscopes in the range of 200–400 kV are becoming more common. In TEM investigations of colloidal systems, voltages between 80 and 200 kV are usually employed. Cryo TEM allows for the direct investigation of colloids in the vitrified hydrated state and information about the internal structure of colloidal system is obtained. By means of a complex sample preparation the formulation microstructure is displayed in its original state and a clear differentiation between nano-sized droplets and other structures can be obtained. Conventional TEM analysis of hydrated systems only provides a limited amount of information since aqueous compounds of the system will evaporate rapidly under vacuum within an electron microscope. Therefore, the development of cryo-electron microscopy of vitrified specimens represented a major progress in this field.

3. Electron microscopy of dried samples

Drying is one of the oldest and cost effective methods of preservation of grains, fruits, vegetables and foods of all varieties. The quality of dried products is strongly dependent on the drying process conditions. Various important characteristics of food change during drying due to loss of moisture from the inner structure to the surrounding environment. Knowledge of microstructure is important in understanding the mechanisms involved during drying of foods. There are studies done in literature investigating the microstructure of dried foods by microscopic techniques. It
is well recognized that changes in many physical characteristics of food during drying are due to changes in the product microstructure. Microscopic analyses were performed to visualize the impact of heating and drying methods on food microstructure in many studies.

3.1 Hot air drying

Hot-air drying is one of the most common drying methods employed for food materials. However, this method has many disadvantages including poor quality of dried products, low energy efficiency and a long drying time. It has been reported that hot-air drying of food materials, involving their prolonged exposure to high drying temperatures, results in substantial deterioration of such quality attributes such as color, nutrient concentration, flavor and texture.

High temperature drying may raise the hydrostatic pressure gradients of moisture between inside and outside of the dried samples and subsequently leads to large voids, resulting in crispy products. Prachayawarakorn et al. used SEM micrographs to characterize the morphology of fresh and dried banana samples at different temperatures. The dried samples became porous while the morphology of reference sample was relatively dense. The evolution of water vapor inside the samples was the reason of pore formation after drying. Pore development depended strongly on drying temperature. Highly porous banana slices were obtained when using the temperatures higher than 120 °C. SEM results showed that it was possible to obtain dried banana slice with less porous at lower drying temperature, particularly at 110 °C. Djanptou et al. employed a novel method for producing ripe mango powder by alternation drying and grinding (ADG) technique. SEM method was used to show evidence of microstructural changes in mango granules during the drying. It was observed that undried mango granules showed an irregular surface structure, while the surface of dried granules is covered by regular forms that can be considered as crystal. So the initial amorphous structure of granules is changed to crystallized structure.

Vega-Galvez et al. studied the effect of temperature and air velocity on the drying kinetics and quality attributes of apple slices during the drying. The hot-air drying process was carried out in a convective dryer and the structural changes of fresh and dried rehydrated apple were observed by cryo-SEM. When comparing these figures with the tissue of raw apple, a clear cell breakage is observed indicating loss of cell content from the breakage zone. There is the least cell damage is least at 40ºC (Fig. 1b) and occurred mainly because of prolonged drying time. The higher air velocity resulted in a more separated and ruptured structure (Fig. 1c).

Jaiboon et al. investigated the changes of cooking and pasting properties as well as starch digestibility of waxy rice during the hot air fluidized bed drying. Fluidized bed drying technique provides good mixing and excellent heat and mass transfer between grains and drying medium. This method not only gives higher drying rate but has also proved to, in some cases, yield dried rice with higher quality. To better understand the effects of drying on the morphology of starch granules, electron microscopy was used. It can be observed that the dried translucent kernel at 150 ºC did not have any starch granules left, while the opaque white kernel still contained some starch granules. These results indicated that starch granule disappearance led to homogenous phase or absence of air spaces inside a kernel, allowing some light to transmit through the kernel, which in turn resulted in the observed translucency. Jaiboon et al. also reported that when waxy rice was dried at 90 ºC, the morphology of most starch granules was rather similar to that of the reference waxy rice although there was some starch gelatinization. The irreversible loss of shape was, however, evident when using drying temperatures of 110 and 130 ºC and some starch granules were fused together. The effect of drying temperature on morphological and chemical properties of two Portuguese Castanea sativa varieties was determined by Correia and et al. It was observed that drying at 70 ºC led to starch granules becoming more shapeless, flattened and rough. Similar results were reported by Attanasio et al. Temperature significantly influences the morphology of starch granules, so it is likely that other properties will also be affected. Mercier et al. studied the effect of fortification with commercial pea protein concentrate on pasta made from durum wheat semolina during the drying. Images showed that pasta microstructure is composed of starch embedded in a protein network. At high temperature, the protein network seemed more dense, rigid, continuous and compact. It was in agreement with the results reported by Zweifel et al. High temperature may cause protein denaturation and promote cross-linking of two gluten proteins, glutenin and gliadin.
3.2 Spray drying

Spray drying is one of the techniques used extensively in food related industries and because very short time of heat contact and the high rate of evaporation, produces a high quality product with relatively low cost. [32,33] This method has several advantages including rapid drying, large throughput and continuous operation. During the drying process, the feed solution is sprayed in droplets in a stream of hot air. The liquid droplets are dried in seconds as a result of the highly efficient heat and mass transfers. [35] The finished product can be made in the form of powder, granules or agglomerates. [36] Scanning electron microscopy images were obtained for spray-dried powders to examine the particle morphology by several researchers.

The effects of various spray drying conditions such as inlet air temperature on the surface composition of spray-dried skim milk powders (SMPs) were investigated by Kim et al. [33]. Scanning electron micrographs of spray-dried SMPs dried at 145 °C and 205 °C confirmed the fast formation of the crust or skin at drying temperature of 205 °C, and at this temperature more lactose than protein appeared at the surface of the powder. SEM images showed that the SMPs prepared using higher feed solids contents were bigger and had a less shrivelled appearance than the SMPs prepared using lower feed solids contents. This confirmed the rapid crust or skin formation at higher feed solids contents. The effect of inlet air temperature on the morphology of milk powders also was investigated by Fang et al. [37]. From the SEM images, it can be observed that lower drying temperature led to relatively uniform size and shape with smooth particle surface, whereas higher drying temperature resulted in size variations and wrinkled particle surfaces.

Spray drying of fruit juices with usual temperatures normally exists in this system; produces a paste like structure instead of powder which sticks to the walls of dryer. This is due to the presence of low molecular weight sugars and acids (citric acid) which have low glass transition temperature (Tg). [38] When the temperature of spray dried particles is 20°C above their glass transition temperature, the molecular mobility of low molecular weight materials becomes high. They are very hygroscopic in their amorphous state and loose free flowing nature at high moisture content. [39] Drying aids, such as maltodextrin, are widely added to the feed to increase the glass transition temperature of dried product and hence overcome the problem of stickiness during spray drying. [35] Fazaeli et al. [40] studied the effect of spray drying conditions (inlet air temperature and compressed air flow rate) and concentration of drying aids (maltodextrin and gum Arabic) on the microstructure of spray dried black mulberry (Morus nigra) juice powders by the use of SEM. The micrographs of the highest and lowest yield are shown in Fig.2. In the microstructure of powders produced with maltodextrin 20DE, it was verified that particles were larger, amorphous, all pilled up and with a strong attraction on each other while when the dextrose equivalent of maltodextrin was decreased or by using gum Arabic, the particles tended to become more spherical, and more scattered.

![Fig. 2 SEM images of black mulberry powder particles produced with: (a) 6% maltodextrin 6DE and 2% gum Arabic at 130 °C, 800 L/h (Max yield), (b) 8% maltodextrin 20DE at 110 °C, 400 L/h (Min yield).](image)

Cano-Chauca et al. [41] studied the effect of different drying aids on the microstructure of mango powder obtained by spray drying. Microstructure analysis obtained by SEM showed that the powder of the obtained mango juices through spray drying using the carriers such as maltodextrin, gum Arabic and starch waxy without the addition of cellulose presented surfaces of amorphous particles and lack of crystalline surfaces. When the concentration of cellulose increased, the particles tended to become more spherical and more scattered.

Spray-drying, also is a well known technology in the food industry that presents the most commonly used microencapsulation method for food ingredients. [42] Non-aqueous extracts of capsicum with high antioxidant activity by using edible vegetable oils as extraction media were prepared by Guadarrama-Lezama et al. [43]. The encapsulation of non-aqueous extracts by spray-drying was conducted to protection of active agents of the extracts against oxidative and deterioration processes. SEM images showed the presence of semi-spherical microcapsules which showed dents and rough surfaces but no evidence of fracture. Krishnan et al. [44] also evaluated the blend of carriers for encapsulation of cardamom oleoresin. Microcapsules from gum Arabic were found to be nearly spherical but had many dents on the surface, whereas microcapsules obtained from maltodextrins and modified starch were partially disrupted. The morphology of powders which were produced with blend of carriers was spherical and had a smooth surface.
3.3 Microwave drying

The application of microwave radiation in drying of foods has become widespread because it minimizes the decline in quality and provides rapid and effective heat distribution in the material. [45, 46] In microwave heating, the heating mechanism differs from conventional methods. Absorption of energy from the microwave field results in internal heat generation, as a consequence internal vapor generation. The internal vapor generation leads to the development of a pressure gradient which significantly increases the rate of moisture transfer as compared to conventional heating methods. Microwaves offer tremendous advantages such as time, space, energy and nutrient savings, in certain food processing operations. [47] Microwaves have been used as a heat source since the 1940s. This technique has been extensively employed in the food and chemical engineering industries. [48, 49]

There are some researches which have shown that combining microwave energy and air drying in a unique way is possible to improve the efficiency and the economics of the drying process. Andres et al. [50] employed a cryo-SEM method to observe the microstructure of apple cylinders which dried by a combined hot air–microwave system. It is observed that air dried samples (Fig. 3a) present a porous structure, and cell walls are greatly shrunk, which leaves wide spaces between neighboring cells. In the micrograph of Fig. 3b two zones can be distinguished, a porous tissue corresponding to the outer zone where the effect of microwave heating occurred to a lesser extent and an inner zone next to the hole, where the tissue appeared more compact due to the combined effect of the high temperatures.

![Cryo-SEM images of apple tissue dried by: (a) air, (b) combining air and microwave.](image)

Increasing microwave power causes an increase in dehydration rate at the second stage. [51] However, too rapid mass transfer could damage the texture in some cases. In addition, non-uniformity of electromagnetic field could create hot spots during microwave drying. In the final stage of drying, product temperature might be increased rapidly to the level that causes scorching. [52] To overcome the limitation of microwave drying, microwave assisted vacuum drying has been used for drying of fruits and vegetables. The advantage is to speed up drying process, increase mass transfer by an increased pressure gradient between inner and outer layers and maintain drying process at low temperature. [53]

In some researches the effects of microwave vacuum drying and hot air drying on food structure were compared by the use of microscopic technique. Therdthai and Zhou [54] employed these two methods for drying of mint leaves and studied the structural morphologies of dried samples by SEM. The microstructure of microwave vacuum dried mint leaves was more porous, open and uniform than that of hot air dried ones (Fig. 4). Increasing microwave power tended to increase evaporation rate, thereby preventing shrinkage and case hardening. Vapor could increase the pressure inside the leaves, as well as enhancing the porosity. This could also explain the improvement in rehydration of dried mushroom by using microwave vacuum drying as reported by Giri and Prasad [55].

![Scanning electron micrograph of dried mint leaves: (a) microwave vacuum drying at 1920W, (b) microwave vacuum drying at 2240W, (c) hot air drying at 70°C.](image)

Based on the scanning electron microscopy (SEM) results, the microstructure of microwave vacuum dried potato was characterized by large porous and irregular structure whereas the microstructure of hot air dried potato by tight packing and strong connection between cells. Therefore, the microwave vacuum dried potato showed higher reconstitution ability during rehydration than the hot air dried potato. [56] Bai-Ngew et al. [57] also compared the structure of microwave vacuum dried with commercially fried durian chips and investigated the effect of freezing prior to drying by scanning...
electron microscopy. Durian is a tropical fruit that possesses a distinct flavor and is nutritionally rich in carbohydrates, protein, fat, phosphorous, iron and vitamin A. The microwave-dried chips had both uniform pore size and distribution. When the durian slices were pretreated by freezing prior to microwave vacuum drying, the dried durian chips had a large porous sponge-like structure.

3.4 Osmotic dehydration

Osmotic treatment is a dehydration technique usually used in fruits and vegetables, which removes moisture from solid food and produces a reduction in food water activity with no change of phase of water and therefore allows storing the foods for longer periods and improving the stability and quality of products. This method is based on the immersion of fruits in a hypertonic solution of sugar. This hypertonic solution presents a higher osmotic pressure and a lower water activity. In the osmotic process, this semi-permeable membrane is represented by the cellular surface structure of the fruits. Food’s weight has reduced by approximately 50% of the original weight due to osmotic dehydration. The structure of the plant tissue can be considered as one of the main factors for understanding the osmotic dehydration process. Castro-Giraldez et al. used low temperature scanning electron microscopy (cryo-SEM) to analyze the structural changes in osmotic dehydration of kiwi fruits. Comparing cryo-SEM micrographs of fresh kiwi and treated ones revealed that extracellular spaces of fresh samples filled with air, while osmodehydrated samples with liquid. Tylewicz et al. also used cryo-SEM to evaluate changes in kiwifruit through the osmotic dehydration treatment with 61.5% (w/w) sucrose solution at short times from 0 to 300 min. It was shown that the water flows from the internal phase of kiwi and the added sucrose is stored in the external and vascular phase. Moreover, the shrinkage of tissue during the treatment can be observed. The effect of sucrose solution concentration on the microstructure of mango cylinders during osmotic dehydration treatment was investigated by Gilardo et al. According to the cryo-SEM micrographs it was observed that in all cases, at the interface, it is difficult to distinguish cells which appear collapsed together with the intercellular spaces. The degree of compactness or collapse of cell structure increases in line with osmotic solution concentration.

Askari et al. investigated the effect of osmotic pretreatment of samples before hot-air microwave drying on the microstructure of dried tomatoes by SEM. The microstructure of samples was affected by microwave treatment at the end of hot air drying. Rapid conversion of microwave energy to heat in the internal parts of samples led to induction of internal pressure which could influence the samples in wide extent. Regarding this fact that at the end of drying process, approximately all open pores were fastened by case hardening phenomenon and water vapor induced by microwave energy could affect the surface during the crossing of superficial layer, so sample’s surface collapse was occurred. The influence of drying method on the fresh samples was strong. The osmotic pretreatment preserved the samples from undesirable structural changes. The surface of treated samples was softer than fresh samples that were treated with only hot-air microwave drying. Figure 5b shows the formation of solute crystals on the samples surface.

Fig.5 SEM images of hot-air microwave dried samples: (a) without pretreatment, (b) osmotic pretreatment.

3.5 Freeze drying

Among the drying methods which are used in food processing industries, freeze-drying is considered one of the most advanced methods for drying high value products sensitive to heat, since it prevents undesirable shrinkage and produces materials with high porosity, unchanged nutrition quality, superior taste, aroma, flavor and color retention, as well as better rehydration properties, superior to those dried with conventional techniques. Freeze-drying is carried out in two stages; the product is first frozen and then the ice is removed by sublimation directly from the solid to the vapor phase. During freeze-drying, ice sublimation causes significant changes in the shape and volume of the food products. Depending on the process conditions, pores or gaps with different characteristics are created by the ice crystals which sublimated. The absence of air prevents product deterioration which causes by oxidation or chemical modification.

Oikonomopoulou et al. used SEM to visualize the microstructure of freeze-dried rice kernels. It was observed that the increase of boiling time leads to increase in both porosity and average pore size. Porosity depends on the water uptake and is higher at longer boiling times where water uptake is higher. In addition, when the absolute pressure in the
freeze-drying chamber is higher, then the porosity is lower compared to the low process pressures. Rhim et al. [67] also employed a field emission scanning electron microscope to investigate the microstructure of freeze dried rice porridge samples. SEM results showed that rigid and porous cube type rice porridges were obtained after freeze dehydration. No shrinkage was observed with all samples. As shown in the SEM images, a porous honeycomb-like structure was observed in all freeze-dried rice porridge samples, which produced mainly by direct sublimation of ice. The freeze-dried rice porridge produced through fast freezing has a denser structure with less pore sizes resulting in harder texture. Chen and Mustapha [68] studied the survival of freeze-dried microcapsules of α-galactosidase producing probiotics in a soy bar matrix. The SEM images of microencapsulated L. acidophilus before and after freeze-drying clearly illustrated well maintained shape and size of probiotic microcapsules through freeze-drying, thus the dried probiotic microcapsules could offer a feasible way to convey the probiotic benefits in food products manufacture. Lee et al. [69] also noticed that freeze-drying method provided a dry product with porous structure as supported by scanning electron micrographs. The results showed that freeze drying method was a good choice for producing antioxidants in food industry.

3.6 Superheated steam drying

Superheated steam drying has been reported as an effective technique for drying purposes because it has many potential advantages. [70] This drying technique is an airless drying which uses high-temperature steam to transport heat to a product, making moisture inside evaporates from the product at its boiling point with no diffusional resistance. [71] These advantages include high quality attributes of dried products, environment-friendly drying, prevention of fire and explosion hazards, low energy consumption and high drying rates under certain conditions. Therefore, superheated steam drying has aroused interest as an alternative technique for meat products drying. [72] Drying with high-temperature superheated steam leads to increased drying rates and effective diffusion coefficients of the drying products. [73, 74]

Potato chips are generally prepared by deep-fat frying thin potato slices and a technique to produce low-oil content chips, with high quality, is required. Recently, superheated steam drying (SSD) has been applied to dry potato chips with various degrees of success. Pimpaporn et al. [75] studied the effects of combined pretreatments on microstructure of potato chips that dried by low-pressure superheated steam drying via scanning electron microscope. SEM photographs showed that the chips dried at 80 and 90 ºC had more uniform pore size and pore distribution compared with the chips dried at 70 ºC. Moreover, more extensive surface shrinkage was found on the samples dried at 70 ºC. Somjai et al. [73] investigated a novel strategy to develop a model for longon drying using a two-stage superheat steam drying and hot air drying process. SEM observation revealed that this method of drying at low steam temperature formed structures containing many small voids with a few large cavities, resulting in considerable pore space inside the sample tissue. Nathakaranakule et al. [71] reported that SEM images of fresh chicken breast clearly showed the muscle fibers and connective tissues while in dried chicken by superheated steam drying muscle fibers were shorten and shrank and the collagenous connective tissue was broken and completely hydrolyzed into gelatin. On the other hand, chicken dried by both combined techniques exposed to the high-temperature environment of SSD shorter than in the case of purely SSD. Therefore, some broken collagenous connective tissues have still remained.

SEM was also used by Jamradloedluk et al. to investigate the microstructure of low fat durian chips that dried via hot air drying and superheated steam drying [76]. As durian was heated for a specific period of time, its middle lamella dissolved and the cell walls were damaged. It was evident that superheated steam dried product had less uniform, fewer but larger pores than hot air dried products (Fig. 6). This is probably due to the fact that the temperature of the material dried in superheated steam sharply rises to 100 ºC; consequently, the water boiled rapidly in durian. The evolution of steam led to development of large pores within the material.

Fig. 6 Scanning electron micrograph of durian cross section of: (a) hot air dried sample, (b) superheated dried sample. [76]

4. Freezing of foods

Freezing is among the most popular and efficient methods of food preservation. The key step determining the efficiency of process and the quality of frozen product is the phase transition part of the freezing process involves the conversion
of water to ice through the crystallization process. In the freezing of foods, the formation of large ice crystals which are mostly extracellular, results in significant damages to the tissue. On the other hand, the formation of fine crystals that are evenly distributed both inside and outside the cells, leads to better preserved quality of the product due to less damages to the tissue. Ultrastructural changes associated with freezing–thawing have been studied in several types of tissue by Partmann, Bomben and King, and Roy et al.

Delgado and Rubiolo employed SEM to observe the microstructure of frozen strawberries. Fig. 7a shows a control sample of strawberry tissue, which did not receive any other treatment but the preparation for SEM. The bright regions in the micrograph are mainly the cytoplasmic membrane and the cell walls; the darker regions are holes where ice and cell contents were before. The structure of a sample frozen at a fast freezing rate (2.43°C/min) is shown in Fig. 7b. The appearance quite similar to fresh sample would indicate that the freezing rate was rapid enough, so that the ice nucleation and crystal growth did not damage the cell walls and ice formation was mainly intracellular. Tissue appearance of Fig. 7c would indicate that extracellular freezing caused tissue shrinkage and cell collapse.

Fig. 7 Scanning electron micrographs of strawberry tissue (a) fresh, (b) frozen at -30°C, (c) frozen at -20°C. [85]

Bomben et al. also used SEM for observation of ice crystals in apple tissue. With the application of power ultrasound during the freezing process the freezing efficiency can be improved and the micro structural properties of frozen foods are better preserved. Increase in heat and mass transfer rates and the initiation of ice nucleation are among other advantages. Sun and Li employed a cryo-SEM to observe the microstructure of potatoes and compare the effect of immersion freezing and ultrasonically assisted immersion freezing. Comparing to fresh samples, the shapes of cells after freezing and thawing became less uniform, instead of polyhedral. Cell wall disruption and large intercellular voids were distinctly viewed, and were much larger than those air spaces observed in an intact tissue. It revealed the presence of extra-cellular formed ice crystals in large size during freezing. The cryo-scanning micrographs for frozen-then-thawed potato tissues under various ultrasonic powers showed no cell wall disruption. This suggested the presence of very small ice crystals inside and outside the cells. As a result, the intercellular spaces did not become enlarged, the plasma membrane remained close to the cell wall and the cell walls did not separate or rupture. The structural integrity was maximally maintained, leading to the high quality of frozen products. Fernandez et al. also employed cryo-SEM method to observe the ice crystals formed during high-pressure shift freezing (HPSF) and high-pressure assisted freezing (HPAF) in gelatin gel samples. Pressure-assisted means the phase transition under constant pressure; pressure-shift means the phase transition due to a pressure release. The micrographs of control gelatin samples presented small, round pores which were homogeneously distributed. These pores were uniform in size. The image of air-blast frozen gelatin sample showed that the resulting ice crystals were large and polygonal shaped, with a high mean equivalent diameter, ranging from 65 up to 311 mm. In HPSF experiments, a large number of small ice crystals were produced whereas in HPAF experiments conducted at identical pressure conditions, the number of ice crystals formed was considerably lower and the crystals were larger.

The damage to dough structure caused by frozen storage can be illustrated through the use of low-temperature scanning electron microscopy (SEM). The dough can be likened to a foam in which gas bubbles are entrapped in the starch/gluten matrix. These bubbles are shown as spherical voids in the electron micrographs of frozen dough. However, the presence of ice crystals formed during freezing, represented by angular voids in the micrographs, can disrupt the foam structure. Zounis et al. studied the effect of temperature and duration of frozen storage and cycling temperature conditions on the microstructure of dough. The major structural changes were the growth of voids and the separation of gluten from starch with increasing storage time. Disruption of dough structure at −10 °C constant storage was primarily due to minor amounts of yeast fermentation. Storage at −20 °C for several weeks resulted in slight structural damage caused by water migration and ice-crystal growth. When subjected to cycling temperature conditions, both yeast activity and ice-recrystallisation may have been factors causing damage to dough structure. Kajak-Siemaszko et al. used electron microscopy analysis to visualize protein aggregate conformation (shape and size). Their analysis confirmed that, frozen meat contained larger protein aggregates. The food industry uses frozen meat as raw material for a variety of meat products, but freezing treatments tend to cause texture damage to real foods due to the large extracellular ice crystals formed.
5. High hydrostatic pressure

The main application of high pressure (HP) in the food industry is for the elimination of microbial pathogens and the extension of shelf-life. The viability of vegetative micro-organisms is affected by inducing structural changes in their cell membrane or by the inactivation of enzyme systems responsible for the control of metabolic reactions. 

Reviews by Manas and Pagan [94], Patterson [95], and Rastogi et al. [96] provide comprehensive summaries of the effects of HP processing on the microbial inactivation and food safety.

The inactivation mechanism by high pressure for yeasts is close to the one for bacteria, in that high pressure affects the cell membrane permeability and cellular structures, which is responsible for protein denaturation. Indeed, a mild high pressure treatment (300 MPa, 15 min, 25 °C) modifies the cell walls and plasma membrane of Saccharomyces cerevisiae, but it seems that the intracellular membrane is the first target in the inactivation process. Goh et al. [99] reported the presence of wrinkles on the cell surface of pressurized S. cerevisiae (600 MPa, 60 s, 60 °Bx) and changes on the shape of cells (600 MPa, 60 s, 40 °Bx).

Marx et al. [100] used microwave assisted dehydration environmental scanning electron microscopy to compare the effect of high hydrostatic pressure (HHP), pulsed electric fields, and thermo-sonication on the structure of Saccharomyces cerevisiae. During conventional sample preparation for electron microscopy, a number of artifacts have been observed during the chemical fixation and embedding techniques used to preserve the membranes of yeast cells. Most of the reports about cell structure using electron microscopy require several hours to obtain meaningful results. However, microwave assisted dehydration has shown excellent preservation of cells and tissues, a considerable reduction of processing time, similar quality in obtained images compared with conventional methods and costs reduction.

In Fig. 8b, the image of pressurized yeast cells is observed with a high degree of damage. The opening and lack of cell wall on the surface of the yeast are the main characteristics observed. The cell wall also features some roughness, and the smooth surface with the granular particles shown in the control cell is no longer observed. Curling of the cell wall is also observed that could be due to cell compression during pressurization and further perforation when the pressure is released. The degree of destruction after pulsed electric fields is not as evident as in the case of high pressure (Fig. 8c). Meanwhile, the cell in center of the image shows the formation of pores on both sides. In Fig. 8d the yeast cells presented a high extent of destruction. The breakdown and perforation of the cell wall is observed in most of cells, showing in some cases the split of the cells exposing the undefined cytoplasm content to the outer environment and high amount of cell debris.

![Fig. 8](image)

Environmental scanning electron microscopy of S. cerevisiae (a) during early stationary phase, (b) after high hydrostatic pressure (600 MPa, 7 min, 21 °C), (c) after pulsed electric fields (30.76 kV/cm, 40 °C, 21 pulses (2 μs)), (d) after continuous thermo-sonication (120 μm, 60 °C, 30 min). [100]

In addition to microbial destruction, it has been reported that HHP offers the dairy industry numerous practical applications to produce microbially safe, minimally processed dairy products with improved performances, and to develop novel dairy products of high nutritional and sensory quality, novel texture and increased shelf life. In relation to cheese texture and microstructure, Buffa et al. [106] by the use of confocal laser scanning microscopy displayed cheeses from HP-treated milk with a regular and compact protein matrix with small and uniform fat globules resembling the structure of cheeses made from raw milk. Those cheeses made from raw or HP- treated milks were firmer and less fracturable than cheeses made from pasteurised milk. Penna et al. [107] studied the effect of thermal treatment, high hydrostatic pressure, and combined treatments of HHP and heat on the microstructure of probiotic low-fat yogurt by SEM. The microstructure of heat-treated milk yogurt was composed of fewer interconnected chains of irregularly shaped casein micelle structures, forming a network that enclosed the void spaces, while the microstructure of HHP treated yogurt exhibited more interconnected clusters of densely aggregated protein with reduced particle size, appearing more spherical in shape and exhibiting a smoother and more regular surface and more uniform size distribution. The combined HHP and heat milk treatments led to compact yogurt gels with increasingly larger casein micelles clusters interspaced by void spaces, and exhibited a high degree of cross-linking. Therefore, the combined HPP and heat treatment before fermentation would be a better process for a uniform consistent microstructure with less physical defects. Knudsen and Skibsted [108] processed milk with high hydrostatic pressure in order to modify the casein
micelles. The images of casein micelle structure in untreated and pressure-treated skim milk were obtained by using cryo-transmission electron microscopy (cryo-TEM). Pressurisation of milk changed the appearance of the casein micelles compared to untreated milk. Some of the large micelles present in milk, after pressurization, had a surface that appeared smoother compared to the casein micelles in untreated milk. Pressurisation of milk increased the number of small particles; however, a fraction of large casein micelles, which appear perfectly spherical, was also present.

High pressure technology is also a typical physical modification way which offers a new possibility for the application of starch in food products. Starch modification is an effective way to improve the functional characteristics of starch. Li et al. studied the effect of HHP on morphological properties of mung bean starch granules by using SEM. The native mung bean granules (Fig. 9a) have typical kidney and ellipse shapes with particle size distribution, ranging from 2 to 30 nm (longer axis). The surface of native starch granules appeared smooth, without pores and fissures. The mung bean starch treated with high pressure (600 MPa/30 min) collapsed and became “doughnut-shaped” as it is shown in Fig. 9b, and it is believed that this was the typical granular structure for pressure gelatinization. 

![Fig. 9 Scanning electron micrographs of mung bean starch granules (a) untreated, (b) HHP treated at 600 MPa.](image)

Native and high pressure treated starch granules were also examined under scanning electron microscopy (SEM) by Liu et al. after high pressure treatment, the shapes of starch granules were changed and the original smooth surfaces became rough. Similar results were reported by Błaszczaż et al., Stolt et al.; Kudta and Tomasiak.

6. Pulsed electric fields

Pulsed electric field (PEF) utilises high intensity electric field pulses to inactivate the microorganisms mainly in liquid foods at relatively low or moderate temperatures (<60°C), whilst preserving the fresh flavour, colour and integrity of heat sensitive components. A typical PEF food processing unit comprises of a high voltage pulse generator, a treatment chamber, a fluid handling system and control and monitoring devices. Depending on particular PEF systems used, typical PEF treatment parameters include pulsed field intensity of 15-50 kV cm⁻¹, pulse width of 1-5 µs, and pulse frequency of 200-400 Hz (pulses/s). Main theory for cell inactivation using pulsed electric fields is irreversible electroporation, as mentioned by many authors. Also, when electric field intensity is very high (40–50 kV/cm), the physical damage is not only limited to membrane disruption, but also homogenization or aggregation of cells can be induced. The destruction of membrane structure due to the interaction between cell and electric field will produce the formation of pores into the cell wall that will allow the free interchange of components from inside of cell with the surrounding media and vice versa, with subsequent cellular death.

The effect of pulsed electric fields on micro-organisms can be observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Some authors have reported the effect of PEF on the ultrastructure of different microorganisms such as L. brevis, S. cerevisiae, Staphylococcus aureus, L. plantarum, L. innocua and E. coli. Evrendilek et al. determined the effects of pulsed electric fields (PEF) on Penicillium expansum inoculated into different fruit juice/nectars based on light and scanning electron microscopy (SEM) observations. Light microscopic observation of P. expansum spores exposed to the pulsed electric fields showed degenerative changes in the morphology relative to spores in control samples and fungal spores appeared to be degraded, and large coagulations were visible within the cell. SEM observations also revealed cytoplasmic coagulation, shrinkage and degradation on morphology of fungal spores after the PEF treatment. Other studies reported similar effects of PEF treatment on the morphology of different microorganisms. According to Pothakamury et al. Staphylococcus aureus cells inoculated into a model food and subjected to 64 pulses at 20, 40 and 60 kV/cm revealed ultrastructural changes based on SEM and transmission electron microscopes (TEM). SEM examination revealed that the cell surface was rough after PEF treatment. Another study conducted for TEM observations of Pseudomonas fluorescens treated by PEF reported that the cell wall was destructed after PEF processing and perforations were formed on the cell membrane, thus causing the cell content to divulge, cell contraction and final cell death.

Morphological changes in L. brevis induced by High-intensity pulsed electric fields (HIPEF) in orange juice were studied by Elez-Martinez et al. by the use of scanning electron microscopy (SEM) and Transmission electron
microscopy (TEM). The SEM of both treated and untreated cells showed the general rod shapes. Untreated cells exhibited a smooth surface, while the cells subjected to HIPEF treatment had a rough surface and some of them are shrunken. The differences between untreated and HIPEF-treated cells were clearer in the TEMs. Untreated control cells exhibited thick cell walls and possessed a well-differentiated cytoplasmic organization. Nevertheless, the cells exposed to HIPEF exhibited thinner or ruptured cell walls and a completely disorganized cytoplasm as well as some cytoplasmatic material had leaked out of the cells (Fig. 10). Thus, damage to the internal organization in HIPEF-treated cells inhibited the growth and reproduction of the cells.

Fig. 10 Transmission electron microscopy images of L. brevis in orange juice (a) untreated, (b) HIPEF-treated. [126]

PEF treatment has been widely used, not only for nonthermal pasteurization, but also for enhancing chemical reactions and modifying large molecules. [127, 128] By scanning electron microscopy, Han et al. [127, 128, 129] studied the effect of PEF on the structure of potato, corn, and tapioca starch, respectively. In all papers, it had been concluded from SEM analysis that dissociation and damage of PEF-treated starch granules appeared. The SEM micrographs of native starch showed that the surface morphology of granules was smooth, oval, and irregularly shaped. After being suffered the PEF treatment, most starch granules still retained their granule shape. However, some roughness or damages emerged on the surface of starch granules and some pits emerged and some small particles aggregated together forming bigger ones, indicating that the granule structure of native starch had been altered by PEF. After the PEF treatment at the highest electric field strengths, starch was significantly deformed. For most starch particles, their granule shape seems to have been lost. Furthermore, some fragments congregated and showed gel-like structures. The newly generated particles were smaller than native starch granules, so they might be the lost envelopes of granules.

Another application of PEF is the possibility of fine regulation of electric power input and result in effective permeability of cellular membranes without significant temperature elevation. [130, 131] Abdullah et al. [132] applied pulsed electric field technology (PEF) to Podophyllum peltatum as a novel approach to enhance the extraction of podophyllotoxin. Podophyllotoxin is valuable for the treatment of cancer and venereal warts. The observation of P. peltatum using scanning electron microscopy revealed that the cell wall was indeed damaged (Fig. 11). The cell constituents were no longer found in the cells and only the cell walls remain and smooth cell walls were deformed due to PEF treatment. In some areas, the smooth structure of the cell wall was no longer observed. They concluded longer PEF treatment time had imposed enough damage on P. peltatum samples to enhance the diffusion of podophyllotoxin.

Fig. 11 Scanning electron micrographs of exterior cells of a P. peltatum (a) untreated, (b) PEF treated. [132]

7. Ultrasound

Ultrasound technology is based on mechanical waves at a frequency above the threshold of human hearing (>16 kHz). These waves travel either through the bulk of a material or on its surface at a speed which is the nature characteristic of the wave and the material through which it is propagating. [133, 134] Ultrasound is propagated via a series of compression and rarefaction waves induced on the molecules of the medium passed through. [135, 136] Alternative methods for pasteurization and sterilization are gaining importance; due to increased consumer demand for new methods of food
processing that have a reduced impact on nutritional content and overall food quality. Ultrasound processing or sonication alone is not very effective in killing bacteria in food; however, the use of ultrasound coupled with pressure and/or heat is promising. Thermosonic (heat plus sonication), manosonic (pressure plus sonication), and manothermosonic (heat and pressure plus sonication) treatments are likely the best methods to inactivate microbes, as they are more energy-efficient and effective in killing microorganisms. [137]

Wordon et al. [138] investigated the effect of hurdle technology using heat and ultrasound, on the structure and metabolic status of *Saccharomyces cerevisiae*. Scanning electron microscopic investigations showed that when compared to the untreated *S. cerevisiae* cells, extensive boundary damage occurred in *S. cerevisiae* exposed to 1 or 5 min of sonication. Micrographs suggested that cell contents from *S. cerevisiae* cells were forcefully extruded from the cells, possibly from increased intracellular pressure, experienced during sonication. Guerrero et al. [139] using TEM to study sonication-induced injury in yeast cells, also showed early cell boundary damage.

Power ultrasound is also found to be able to improve the quality of the frozen product. Cryogenic scanning electron microscope photos indicate that plant tissues of ultrasound-assisted frozen potatoes exhibit a better cellular structure as less extracellular void and cell disruption/breakage appear than those without acoustic treatment. [140] This might also be due to that cavitation bubbles could have induced the occurrence of intracellular nucleation, which usually is not able to occur due to insufficient degree of supercooling, although cavitation bubbles can help to reduce crystal size, minimise cell dehydration and maintain product original shape. [141]

The structural features of ultrasonic treated potato starch granules were investigated by Zhu et al. [142] by the use of scanning electron microscopy. The native starch granules showed the smooth surface. When starch samples were treated with ultrasound, the notch and groove appeared in the surface. It is apparent that the damage was aggravated as ultrasonic power increasing. The slight “scratch” was gradually replaced by more obvious erosion. The formation of erosion and cavity provided more channels for water diffusion into starch granules, which would change the granular structure of the potato starch. Jiang et al. [143] also used scanning electron microscopy to investigate the effect of ultrasonic–microwave synergistic treatments on the structure of rice starch gels. The results showed that rice starch gels prepared by ultrasonic–microwave synergistic treatments had smaller holes and larger connection parts compared to conventionally heated one.

Another application of ultrasound is meat tenderization and increasing salt diffusion in meat immersed in brine. [144, 145] The effect of ultrasonic assisted curing technology on porcine tissue microstructure was investigated by Siró et al. [146]. SEM revealed microstructural changes in porcine muscle for all types of brining methods. Swelling of fibers and increasing in the thickness of the filaments was observed due to ultrasonic treatment. Increased interfilament space permits muscular water uptake, thus causing improved tenderness. The influence of ultrasonic treatment on tissue microstructure can be explained by cavitation, which supports the charging of protein membranes of myofibrils. It was also noticed based on the TEM micrographs (Fig. 12), that myofibrils were ruptured along with the z-lines due to ultrasonic cavitation. Similar observations were made by Got et al. [147] who applied ultrasound for the tenderization of bovine *Semi-membranosus* muscle.

![Fig. 12 TEM micrographs of pork loins showing rupture of myofibrils along with the z-lines. (a) Static brined; (b) ultrasound treated.](image)

Ultrasound is also used as an inexpensive, reproducible, simple and efficient alternative method of industrial relevance to improve the extraction process of food bioactives. All the mechanical effects involved in ultrasound can accelerate the eddy and internal diffusion giving rise to an increased mass transfer [148] and they allow a greater penetration of solvent into the sample matrix [149]. Karki et al. [150] investigated the use of ultrasound prior to soy protein extraction to simultaneously enhance protein and sugar release in the extract. SEM images showed several micro-fractures appeared in the soy flakes following ultrasound pretreatment. The severity of disintegration improved progressively with increase in amplitude and sonication time. At high amplitude, there was near complete rupture of defatted soy flakes cell with large numbers of fragmented cell matter. SEM study showed particulate surface material with a sponge-like texture rather than prominent protein bodies. The effect of ultrasound on the extraction of polysaccharides from the mulberry leaves were studied by Ying et al. [151]. The microstructure of tissues of the mulberry leaves after extractions were observed by SEM image. The results showed that the cell walls in tissues of the mulberry leaves.
leaves were explosively damaged after treated ultrasound assisted extraction and many hollow openings led to explosive disruption of the physical structure in cell walls of tissues.

8. Conclusion

Various common microscopic techniques have been reviewed and their potentials for use in the food industry discussed. Clearly, the most appropriate technique depends on the property of the sample to be monitored, the nature of the sample and its environment and other practical restrictions. Electron microscopy techniques are an essential tool to obtain precise information about basic structural properties of different kinds of foods. The effects of processes such as different kinds of drying (hot air drying, spray drying, microwave, osmotic drying, freeze drying and superheated steam drying), freezing, high hydrostatic pressure, pulsed electric fields, and ultrasound on foods microstructure are reported. This review has shown that these image capture techniques can be used to find the relationship between food processing conditions and morphological changes of the food components. As the structure of foods can have a profound influence on its nutritional value, the proper conditions that help to retain the quality of the product can be defined by using current microscopic techniques.

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