VISUALIZATION OF INTRACELLULAR ICE CRYSTAL FORMATION USING X-RAY MICRO-COMPUTED TOMOGRAPHY

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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of degree of Master of Engineering in Bioprocess.

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I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Special dedication of this grateful feeling to my…

understanding husband;
Mr. Mohd Shuadi Alias

lovely son;
Ahmad Aniq Ar-Rayyan

beloved mother;
Mrs. Mek Yah Ahmad

loving brothers and sister;
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supportive families;
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ABSTRACT

This study demonstrates the capability of X-ray Micro-computed Tomography (XMT) technique to characterise the internal ice crystal microstructure of freeze-dried samples (strawberry). The method requires the fruit being frozen under specific freeze-drying temperature of the samples to remove frozen water before scanning to indicate ice crystal and internal structure of the samples. Results are presented for the 2-D ice crystals formed within the samples. The dendrite spacing (size, volume and width) of ice crystals is related to freezing condition of the samples. Only 1% small intercellular voids distribution of the total 99% volume in fresh strawberry samples as compared to 72% void or pore distribution in freeze-dried samples. Other than that, the average width size of ice crystal for two different operating freezing (-20^{o}C and -80^{o}C) temperatures for strawberry samples was 0.15 to 0.29 mm and 0.29 to 0.40 mm respectively. The overall results indicate that the ice crystal distribution within samples were diverse with the axial distance of the material from its cooling surface. The ice crystal size is bigger when the fruits were far from the cooling surface. At the investigated conditions, the comparisons with air-drying technique had been done on jackfruit and the study on those fruits had been limited to image visualization for validation and nutrient analysis. Samples dried in the freeze dryer were seen to retain their shape much better than air dried samples which underwent shrinkage and change in color. Samples dried in freeze dryer possessed less dense structures and consequently displayed more favorable rehydrated textural properties than the air-dried equivalents. Furthermore the total phenolic (TP), and ascorbic acid (AA) of the fresh and freeze-dried samples had been investigated. This method had preserved 3.65 mg/mL AA concentration in freeze-dried strawberry samples with ultrasonic pre-treatment and no AA had been detected in the air-dried strawberry samples. Total phenolics (TP) concentrations was also evaluated and compared to the TP content in fresh samples that were frozen and stored at -20^{o}C. The average TP content of frozen, freeze-dried and air-dried strawberries are 270.5, 231.0, and 28.7 mg/100 g of fresh weight, respectively.

ABSTRAK

Kajian ini menunjukkan kemampuan teknik X-ray mikro tomografi (XMT) untuk mencirikan secara mikro ais kristal yang terbentuk di dalam sampel beku-kering (strawberi). Kaedah ini memerlukan sampel buah yang dibekukan di bawah suhu beku-kering tertentu untuk menghapuskan kesan ais sebelum imbasan untuk menunjukkan hablur ais dan struktur dalaman sampel dijalankan. Keputusan dipaparkan secara 2-D untuk ais kristal yang terbentuk di dalam sampel buah beku-kering. Jarak dendrit ais kristal (saiz, isipadu dan luas) dipengaruhi oleh kaedah pembekuan terhadap buah-buahan. Hanya 1% ruang interselular pengedaran didapati dalam 99% jumlah sampel (strawberry segar) berbanding dengan ruang 72% pengedaran pori di dalam sampel beku-kering (strawberry). Selain itu, saiz ais kristal bagi sampel yang dibekukan pada -20^{o}C dan -80^{o}C sebelum menjalani beku-kering teknik adalah 0.15-0.29 dan 0.40-0.29 mm. Keputusan keseluruhan menunjukkan bahawa pelbagai bentuk taburan ais kristal di dalam sampel berbeza mengikut jarak sampel dari permukaan pendinginnya. Saiz ais kristal lebih besar apabila sampel buah berada jauh dari permukaan pendingin. Pada keadaan diselidiki, perbandingan dengan pengeringan secara pemanasan dijalankan ke atas buah nangka dan keputusan kajian adalah terhad kepada paparan imej dan analisis nutrien sahaja. Sampel yang dikeringkan menggunakan alat beku kering ternyata jauh lebih baik untuk mempertahankan bentuk asal berbanding pengeringan secara pemanasan dimana ia mengalami perubahan penyusutan bentuk dan warna. Sampel yang dikeringkan dalam alat beku kering mempunyai struktur kurang padat berbanding pengeringan secara pemanasan. Selain itu, kandungan total fenol (TP), dan asid askorbat (AA) sampel segar dan beku-kering telah dianalisis. Keputusan telah menunjukan 3.65 mg/ml AA masih terkandung dalam sampel beku-kering menggunakan kaedah ultrasonik dan AA tidak dapat dikesan di dalam sampel yang melalui pengeringan secara pemanasan. Kandungan TP pada sampel segar yang dibekukan dan disimpan pada-20^{o}C juga dibandingkan. Purata kandungan TP bagi teknik secara beku, beku-kering dan pengeringan biasa buah strawberi adalah 270.5, 231.0, dan 28.7 mg/100 g.

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LIST OF ABBREVIATIONS

Micro-CT	Micro-computed tomography
CTan	Skyscan tomographic analysis package
°C	Degree Celsius
°F	Degree Fahrenheit
2-D	Two-dimensional
3-D	Three-dimensional
mm	Millimetre
g	Gram
µA	Current(microampere)
kV	Voltage(kilo volt)
mTorr	Pressure (mili Torr)
%	Percentage
g/ml	Concentration(gram/millilitre)

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Improving methods of food preservation is an essential technique needed by all in the food industry. Achieving the right preservation technique is very important in relation to the changes of nutritional and sensory qualities of food. Freezing is a conventional food preservation technique in which the temperature of food is reduced below its freezing point and a proportion of the water undergoes a change in its state to form ice crystals. The immobilization of water to ice and resulting concentration of dissolved solutes in unfrozen water lowers the water activity of the food (Fellows, 2000). This method often results in substantial textural damage caused by the growth of ice crystals within the delicate structure either present naturally or created during processing (Mousavi et al., 2005).

The preservation of biological products by reducing their water content can be achieved by several dehydration techniques. The underlying principle of drying foods is to lower the moisture content in order to reduce water activity and prevent spoilage. Water activity is a critical factor that determines shelf life, with most bacteria unable to grow below a value of 0.91 and moulds cease to grow below 0.80 (Brown et.al., 2008 and Beuchat, 1983).Water activity also plays a significant role in determining the activity of enzymes and vitamins in foods and can have a major impact on their colour, taste and aroma. Additionally, moisture removal reduces the weight and the bulk of food products to facilitate transport and storage. While imparting these benefits, loss of moisture during drying may also inflict undesirable effects on the product's microstructure (Brown et al., 2008). This is unfortunate given that food structure

influences nutritional availability, chemical and microbiological stability, texture, physical properties and transport properties (Aguilera, 2005; Aguilera et al., 2003; De Roos, 2003 and Aguilera et al., 2000).

Among these methods, freeze-drying is considered as the reference process for manufacturing high-quality dehydrated products. This drying process involved a preliminary freezing of the products followed by placing them under reduced pressure (< 300 Pa) with a sufficient heat supply to sublimate ice (2800 J per gram of ice). Compared to classical dehydration techniques, the main advantages of the freeze-drying process are: (i) the preservation of most of the initial raw material properties such as shape, appearance, taste, colour, flavour, texture, and biological activity and (ii) the high rehydration capacity of the freeze-dried product (Hammami and Rene, 1997). Freeze-drying is a technique that results in high-quality dehydrated products due to the absence of liquid water and the low temperatures required in the process. The solid state of water during freeze-drying protects the primary structure and minimizes changes in the shape of the product, with minimal reduction of volume (Ratti, 2001). In addition, it contributes to preserve constituents such as minerals and vitamins, as well as to retain original flavour and aroma (George et al., 2002).

Freeze-drying appears, therefore, as a promising technique for dehydration of thermal-sensitive materials, such as strawberries and jackfruits. The final quality of dried fruits can be affected by the physical and structural changes that occur during drying, which may include deformation in terms of shrinking. The true and apparent densities, as well as the porosity of the material define the rehydration capacity of a dried product. The drying process may alter these properties, resulting in products with modified texture, optical, thermal and nutritional properties (Krokida et al., 2000).

Another parameter that can be used as a quality index of nutrients during food processing and storage is the ascorbic acid content, due to it being an unstable constituent, sensitive to variations on pH, temperature, moisture content, oxygen and light. If the ascorbic acid is retained after processing, other nutrients are likely to be retained. Lin et al. (1998) performed a comparative study among vacuum microwave, hot air and freeze-dried carrot slices. They observed that freeze-dried carrots did not

present significant losses in the content of Vitamin C, while the samples dried by hot air and vacuum microwave presented losses of 62% and 21%, respectively. Luanda et al. (2006) and Shadle et al. (1983) also investigated the Vitamin C content of carrots after convective and freeze-drying, reported losses of 81.3% and 60.8%, respectively in the dry samples.

The rehydration ratio can be considered as a measure of the injuries caused by the processing and drying to the material (Krokida et al., 2003). It is generally accepted that the rehydration capacity is dependent on the degree of cellular and structural disruption. During drying, Jayaraman et al. (1990) observed irreversible cellular rupture and dislocation, resulting in loss of integrity and hence, in a dense structure of collapsed, greatly shrunken capillaries with reduced hydrophilic properties, which are reflected by the inability to imbibe sufficient water to fully rehydrate.

This study is concerned within the freeze-drying for strawberry. The aim was to determine the influence of processing conditions on the ice crystal formation in those fruits by using the application of X-ray Micro-computed Tomography (XMT) techniques. Ice crystal growth in fruits has been found to extensively damage those fruits. Samples of strawberry were selected because they are highly perishable fruit with very short life span (not more than 4 to 5 days in refrigeration conditions). In spite of the importance of knowing the ice crystal formation of these freeze-dried fruits, detailed information on visualization is still lacking in the literature, particularly for frozen fruits. The XMT techniques applicable in this study had been done by collaborated with Malaysian Nuclear Agency (MINT) in which provided the equipment for data collection. The purpose of this work is also to determine quality parameters, such as the ascorbic acid (AA), total phenolic(TP), the glass transition temperature, the water activity and the rehydration capacity for freeze-dried samples and to investigate the effect of dehydration conditions on these properties. The nutrient analysis had been done on collaboration with CEPP, Univesiti Teknologi Malaysia and SIRIM QAS International Sdn. Bhd.

1.2 PROBLEM STATEMENT

A major problem with freeze-drying process is the preliminary freezing of the product will stiffen its structure and subsequently prevent solute and liquid motion during freeze-drying (Hammami and Rene, 1997, and Levine and Slade, 1989). During the formation of ice crystals, they grow and create a uniform network throughout the product that after sublimation yields a dense, spread and homogeneous porous matrix.

Chemical and enzymatic reactions will thus be significantly limited and the phenomena of aroma loss and vitamin degradation will be reduced in comparison to classical drying techniques (Hammami and Rene, 1997, and Simatos et al., 1974). The sublimation phenomenon (direct change from ice to vapour) explains the reason freeze-dried products adulterate a little or not at all and can rehydrate instantaneously. The poor quality and alterations of freeze-dried products that are sometimes encountered are generally linked to the quality of the raw material (nature and degree of ripeness) and to processing conditions (operating pressure, heating temperature, freezing rate, freeze-drying process control) (Genin and Rene, 1996).

Previously a number of research efforts had been reported on the ice crystal formation in frozen food (such as fish, meat and mycoprotein) that may affect their textural, microstructural and qualitative changes. The study on quality of freeze dried strawberry pieces had been done by Hammami and Rene (1997). They had conducted an experiment on a thick layer of strawberry pieces in different operating conditions of freeze drying. Researchers found that working pressure and heating plate temperature during freeze drying were the most important factors affecting the criteria of the final product quality in terms of its appearance, shape, colour, texture and dehydration ratio. The researchers had found the optimal conditions for freeze drying process for strawberry was at 30 Pa, 50^{o}C and the time ranged for freeze drying was from 60 to 65 hours. Although the researchers had investigated the freeze drying time, appearance and colour of the freeze dried strawberries, there was no qualitative and quantitative information about ice crystal formation of these strawberries in thick samples.

Recent studies visualized the ice crystal structures formed during freezing of a number of foods had been applied using X-ray micro-computed tomography (XMT) (Mousavi et al., 2007). An understanding of the relationship between the freezing conditions and the size of ice crystals formed is critical in controlling product quality and texture (Mousavi et al., 2007). The observation of the ice crystals size can be direct or indirect. Direct observation of ice crystal can be done by cryo-scanning electron microscope (Russell et al., 1999), cold microscopy (Donhowe et al., 1991) and confocal laser scanning microscopy (Evans et al., 1996).

While the indirect method such as freeze substitution (Bevilacqua et al., 1979, and Martino and Zarizky, 1998), freeze fixation (Miyawaki et al., 1992) and freeze drying techniques (Woinet et al.,1998a, b; and Fayadi et al.,2001) which followed by the sectioning had also been used. However, the indirect methods assumed the original morphology is maintained during the sectioning into thin enough layers to allow microscopic methods to be used (Mousavi et al., 2005).

Freeze drying is a well established process for the indirect method to observe ice crystals formation and preserve the food products. Freeze-drying is known to produce products of excellent quality, allowing significant structural preservation (Brown et al., 2008 and Sinesio et al., 1995). Indeed it has been reported that the products of freeze-drying are of higher porosity than those of air-drying (Brown et al., 2008; Karathanos et al., 1996; Marabi and Saguy,2004, and Rahman et al., 2002) and other drying methods such as microwave (Brown et al., 2008 and Tsami et al., 1999), and vacuum-drying (Rahman et al., 2002).

X-ray micro-CT (XMT) is relatively a new technique which has found potential applications in food science research and quality evaluation (Mendoza et al., 2006). An axial and lateral resolution down to a few micrometers and without sample preparation and chemical fixation of the architecture of cellular materials can be visualized and analyzed through this technique (Dalen et al., 2003). Studies had been done using XMT. They were firstly studies on the internal microstructure for ice crystal visualization of mycoprotein, carrot, meat, fish, chicken, potato and cheese (Mousavi et al., 2005, 2007), secondly studies to detect internal quality changes in peaches (Barcelon et al.,1999),

thirdly investigation on core breakdown disorder in 'Conference' pears based on their mass density variations during storage (Lammertyn et al., 2003) and lastly quantitative analyze and characterization of apple tissue to micrometer resolution (Mendoza et al., 2006). X-rays are short wave radiations, which can penetrate through fruit (Mendoza et al., 2006). The level of transmission of these rays depends mainly on the mass density and mass adsorption coefficient of the material (Mendoza et al., 2006 and Salvo et al., 2003). The density of many fruits increases with maturity (Mendoza et al., 2006 and Baoping, 1999).

The motivation of those studies was found in the necessity to extract realistic and statistical 2-D internal data for the ice crystals formation in freeze-dried strawberry at micron resolution and thus validate those results by using another fruits for comparison and jackfruit had been selected in order to make a comparison of micro structural evaluation and nutrient analysis with strawberries. Observing the ice crystals formation of the internal microstructure of food is important as it will help in preserving the texture quality of the freeze-dried strawberry and jackfruit.

The criterion of quality is becoming progressively more important in consumer choice. Thus, industrial products and ingredients must offer different convenient properties (tasty, healthy, and safety) that are close to those of fresh product. At the same time, new market demands are emerging that could concern freeze-dried products, for example dehydrated fruits to add in cereals, cereal bars, ice creams, or pastries.

1.3 OBJECTIVE

The main objectives of this study are:

i. to investigate the potential of freeze-drying techniques for strawberry and jackfruit according to the experimental set up,
ii. to compare freeze-dried and conventional air-drying techniques on those fruits and the results were subjected only to image validation using XMT and nutrient analysis,
iii. to employ the application of XMT techniques in order to study the extent of the damage produced by ice crystal growth in freeze-dried strawberry fruit tissues in more details and,
iv. to analyze the nutrient constituents of those freeze-dried fruits. The constituents of ascorbic acid (AA), total phenol (TP), colour, appearance and rehydration had been investigated in those fruits.

1.4 SCOPE

The research is based on experimental studies of freeze-drying technique for fruits by using strawberry. In order to achieve the objectives mentioned earlier, five scoped had been investigated:

i. freeze-drying of strawberry at various operating conditions according to the experimental design: A study of different thickness (5mm, 10mm and 15 mm), ultrasonic treatment, and normal refrigeration freezer under 4^{o}C, freezing at -20^{o}C and -80^{o}C,
ii. comparison of freeze-drying with the conventional air-drying technique: using jackfruit as compared fruit and the result were evaluated only on image visualisation for ice crystal validation using XMT techniques and nutrient analysis.
iii. a study on MR images analysis from previous study in comparison with XMT image analysis in these studies.

iv. a micro-computed tomography scanner is used as characterisation device for the porosity changes while freeze- and air-drying is taking place. XMT device had been provided by Malaysian Nuclear Agency (MINT) for the use of data collections. This scanner uses up-to-date scanning techniques to take x-ray photographs of the sample, while the latter is rotated perpendicularly about its long axis. Reconstruction software then recreates the photographs as circular planes and the extent of drying on the surface of the strawberry sample can be assessed by the amount and size of the pores on its surface for different applicable pre-treatment, thickness and nutritional availability of the strawberry samples, and,

v. nutrient analysis of the fresh and freeze-dried or rehydrated strawberries and jackfruits (total phenol, and ascorbic acid content) using ultra violet (UV) spectrophotometer and high pressure liquid chromatography (HPLC) analysis. This work is also to determine some other quality parameters, such as the glass transition temperature, the water activity and the rehydration capacity for freeze-dried strawberries and to investigate the effect of dehydration conditions on these properties. Some parts of the evaluation (nutrient composition) had been done by collaboration with CEPP Universiti Teknologi Malaysia and SIRIM QAS International Sdn. Bhd.

1.5 SIGNIFICANT OF THE STUDY

Malaysia is one of the fruit producers, consumers and exporters of strawberry and jackfruit. Like other fruits, strawberry and jackfruit may be commercialized in their natural form, frozen pulps or processed juices. The typical weather characteristics that pre-dominate the northeast region in Malaysia with high relative humidity and temperatures however are not favourable to fruit preservation under natural conditions. Particularly for fruits with high moisture contents (in the case of strawberry, the average value is about 91%w.b.), rapid deterioration is commonly observed after cropping. The study also will offer an alternative method for preservation of the fruits and their original constituents. Other than that, special attention has been directed to the development of adequate drying techniques. Besides aggregating commercial value to

the fruits, drying reduces wastes and post-harvest losses, and might allow their commercialization for extended periods, with minor dependence on seasonal conditions.

Freeze-dried preservation retains the nutrient quality of agricultural products over long storage periods. As a method of long-term preservation for fruits and vegetables, freeze-dried is generally regarded as superior to canning and dehydration, with respect to retention in sensory attributes and nutritive properties (Fennema, 1973). The research in this field will be very useful to the frozen industry. The need for freeze-dried food products had become one of the most in demand technologies in food preservation. The significances of this study are:

i. Strawberry and jackfruit itself had been selected as the main raw material in this study due to the existing of many natural antioxidants properties in those fruits and thus giving more health benefits to the consumers. As an example, ellagic acid that was mostly found in strawberry had been useful antioxidant to prevent cancer. Other than that, there is still no added value to those fruit in our local market as an example to produce food product with existing of this natural fruits.

ii. It is important to study the composition of different foods in order to understand the nature of those foods. The nature of the food like color, texture, consistency and quality depends on the constituents that contain in it. This study will help in eliminates the use of synthetic coloring and flavoring agent in food products by introducing the freeze-dried techniques that can help in preserving the initial properties in foods.

iii. Understanding the changes that occur in food during storage, preparation and processing. Food is exposed to different conditions during storage, preparation and processing and these changes may either be desirable or undesirable. The knowledge of undesirable changes during storage, preparation and preparation will enable us to develop and use ideal methods of food storage, preparation and processing which would retain the nutrients to the maximum along with increasing the acceptability.

iv. The study also aiming to offer alternatives fruits preservations which is freeze-drying technique that can make the process more economically (no chemical added to the raw material in this continous processes and

produce products that retains most of its initial properties), environmental and consumer friendly (no more waste from fruits and have fruits on their diets plans) and the design is compact and easy to operate in the food industry.

v. Other than that, the significance of having new types of fruits products which can give longer shelf life, reduced weight for storage, shipping and handling become easier in order to commercialize this new product (freeze-dried) fruits. Our food industry will no more dependable on the seasonal condition and thus will help them in producing new foods (cereals, snack bars) which includes the original fruit taste that can change the consumer bad eating habit to have a portion of fruits in their diet plans.

vi. XMT also can contribute in designing optimized production for freeze-drying products as it will help in observing at micron resolution the internal microstructure of the food products in order to give more preferable freeze-dried products.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The prime interest in the literature study was the use of x-ray micro-computed tomography (XMT) techniques in analyzing the influence of processing conditions (working pressure and heating temperature) on freeze-drying time and product microstructure and, to a lesser extent, the shelf-life of the freeze-dried products and the changes in some properties during storage (Hammami and Rene, 1997; Roos, 1987, and Paakkonen and Mattila, 1991). Significant progress was reached in the understanding of the phenomena occurring during freeze-drying (Hammami and Rene, 1997; Monteiro-Marques et. al., 1991; Rutledge et al., 1994, and Genin and Rene, 1996), quality improvement of freeze-dried vegetables (Hammami and Rene, 1997; Le Loch et al., 1992; Kompany and Rene, 1993; Genin and Rene et al., 1996) or the monitoring and controlling of the freeze-drying process (Hammami and Rene, 1997; Rene et al., 1993, and Roy and Pikal, 1989).

In some cases, improvement (energy saving and quality assessment) of classical dehydration techniques such as hot air drying, fluidized bed drying, vacuum drying, and spray-drying (Hammami and Rene, 1997, and Mujumdar, 1987), as well as the development of new techniques such as 'puffing' (Hammami and Rene, 1997, and Guimard, 1994) had given rise to cheaper products that can be used in place of freeze-dried products. As a result, the challenges and opportunities for the freeze-drying technique require: (i) improvement of the quality of dehydrated products, and (ii) the processing of other products that are technically difficult to dehydrate at high temperature, as an example because of their high sugar content.

2.2 APPLICATION OF PRESERVATION TECHNOLOGY IN FOOD INDUSTRY

Food technology may be defined as a controlled attempt to preserve, transform, create or destroy a structure that has been imparted by nature or processing (Aguilera and Stanley, 1999). Other than that it is the application of science to the commercial processing of foodstuffs. Food is processed to make it more palatable or digestible, for which the traditional methods include boiling, frying, flour-milling, bread-, yoghurt-, cheese-making and brewing (Peter and Julian,1999). Food is also being processed to prevent growth of bacteria, moulds, yeasts, and other microorganisms. It is preserved from spoilage caused by the action of enzymes within the food that changes its chemical composition, resulting in changes in flavor, odour, colour, and texture of the food (Gary, 2008). These changes are not always harmful or undesirable; examples of desirable changes are the ripening cream in butter manufacture, the flavoring development of cheese, and the hanging of meat to tenderize the muscle fibres. Fatty or oily foods suffer oxidation of the fats, which makes them rancid (Peter and Julian, 1999).

Preservation enables foods that are seasonally produced to be available all year long. Traditional forms of food preservation include salting, smoking, pickling, drying, bottling, and preserving in sugar (Zeuthen and Bogh-Sorensen, 2003). Modern food technology also uses many novel processes and additives, which allows a wider range of foodstuffs to be preserved. All foods undergo some changes in quality and nutritional value when subjected to preservation processes. No preserved food is identical in quality compared to its fresh counterpart; hence only food of the highest quality should be preserved.

In order to grow, bacteria, yeasts, and moulds need moisture, oxygen, a suitable temperature, and food. The various methods of food preservation aim to destroy the microorganisms within the food, to remove one or more of the conditions essential for their growth. Adding large amounts of salt or sugar reduces the amount of water available for microorganisms to thrive because the water tied up by these solutes cannot be used for microbial growth (Barbosa-Canovas et al., 2008). This is the principle in

salting meat and fish, and in the manufacture of jams and jellies. These conditions also inhibit the enzyme activity in food. Preservatives may also be developed in the food by controlling the growth of microorganisms to produce fermentation that make alcohol, acetic or lactic acid. Examples of food preserved in this way are vinegar, sour milk, yoghurt, sauerkraut, and alcoholic beverages (Peter and Julian, 1999, and Zeuthen and Bogh-Sorensen, 2003).

2.3 PRESERVATION TECHNOLOGY

Preservation enables foods that are seasonally produced to be available all year long. There are various types of preservation technology in food industry.

2.3.1 Refrigeration

At 5^{o}C (41^{o}F) or below 3^{o}C (37^{o}F) it will help in reducing spoilage for cooked foods, but it is less effective for foods with high water content. This process cannot kill microorganisms, nor stop their growth completely, and a failure to realize this limitation causes many cases of food poisoning. Refrigerator temperatures should be checked periodically as the efficiency of the machinery declines with age as higher temperature will be dangerous for the consumption of the refrigerated food. (Peter and Julian, 1999)

2.3.2 Deep Freezing

Deep freezing (-18^{o}C or below) stops almost all spoilage processes, except residual enzyme activity in uncooked vegetables and most fruits, which are blanched (dipped in hot water to destroy the enzymes) before freezing (Cano and Barta, 2008). Microorganisms cannot grow or divide while frozen, but most remain alive and can resume activity once defrosted. Preservation by freezing works by rendering water in foodstuffs unavailable to microorganism by converting it to ice. Some foods are damaged by freezing, notably soft fruits and salads, the cells of which are punctured by ice crystals, leading to the loss of crispness (Cano and Barta, 2008). Fatty foods such as cow's milk and cream tend to separate.

Freezing has little effect on the nutritive value of foods, though a little vitamin C may be lost during blanching process in fruits and vegetables. Various processes are used for deep freezing foods commercially (Peter and Julian, 1999).

2.3.3 Pasteurization

It is used mainly for milk. By holding the milk at 72^{o}C or 161.6^{o}F for 15 seconds, all disease-causing bacteria can be destroyed (Doona and Feeherry, 2008). Less harmful bacteria survive, so the milk will still go sour within a few days (Peter and Julian, 1999).

2.3.4 Ultra-heat Treatment

It is used to produce UHT milk. This process uses higher temperatures than pasteurization, and kills all bacteria present, giving the milk a longer shelf life but altering the flavor (Doona and Feeherry, 2008).

2.3.5 Drying

It is effective because both microorganisms and enzymes need water to be active. This is one of the oldest, simplest, and most effective ways of preserving foods. In addition, drying concentrates the soluble ingredients in foods, and this high concentration prevents the growth of bacteria, yeasts and moulds. Dried food will deteriorate rapidly if allowed to become moist, but provided they are suitably packaged; products will have a longer shelf life. Traditionally, foods were dried in the sun and wind, but commercially today, products such as dried milk and instant coffee are made by spraying the liquid into a rising column of dry, heated air; solid foods, such as fruit, are spread in layers on a heated surface (Peter and Julian, 1999).

2.3.6 Freeze-drying

This technique is carried out under vacuum. It is less damaging to food than straight forward dehydration in the sense that foods reconstitute better. It is used for quality instant coffee and dried vegetables. The foods are fast frozen, and then dried by converting the ice to vapor under very low pressure. The foods lose much of their weight, but retain the original size and shape. They have a sponge like texture, and rapidly reabsorb liquid when reconstituted (Hamammi and Rene, 1997). Refrigeration is unnecessary during storage; the shelf life is similar to dried foods, provided the product is not allowed to become moist. The success of the method is dependent on a fast rate of freezing, and rapid conversion of ice to vapor. Hence the most acceptable result is obtained with thin pieces of food, and the method is not recommended for pieces thicker than 3cm. Fruit, vegetables, meat, and fish have proved to be satisfactory. This method of preservation is commercially used but the products are most often used as constituents of composites dishes, such as packet meals (Peter and Julian, 1999).

2.3.7 Canning

It relies on high temperatures to destroy microorganism and enzymes. The food is sealed in a can to prevent contamination. The effect of heat processing on the nutritive value of food is variable. For instance, the vitamin C content of green vegetables is much reduced, but owing to greater acidity in fruit juices vitamin C is quite well retained. There is also a loss of 25-50% of water soluble vitamins if liquor is not used. Vitamin B (thiamine) is easily destroyed by heat treatment, particularly in alkaline conditions. Acid products retain thiamine well, because they require only minimum heat during sterilization(Tewari and Juneja, 2008). The sterilization process seems to have little effect on the retention of vitamins A and B2. During storage of canned foods, the proportion of vitamin B and C decrease gradually. Drinks may be canned to preserve the carbon dioxide that makes them fizzy (Peter and Julian, 1999).

2.3.8 Pickling

It utilizes the effect of acetic (ethanoic) acid, found in vinegar, in stopping the growth of moulds. In sauerkraut, lactic acid produced by bacteria, has the same effect. Similar types of non harmful, acid-generating bacteria are used to make yoghurt and cheese (Peter and Julian, 1999).

2.3.9 Curing of Meat

It involves soaking in salt (sodium chloride) solution, with saltpeter (sodium nitrate) added to give the meat its pink tinge and characteristic taste. Bacteria convert the nitrates in cured meats to nitrites and nitrosamines, which are potentially carcinogenic to humans (Peter and Julian, 1999).

2.3.10 Irradiation

It is a method of preserving food by subjecting it to low-level radiation. The exposure of food to low-level irradiation kills microorganisms. Irradiation is highly effective, and does not make the food any more radioactive than it is, naturally. Irraddiated food is used for astronauts and for immunocompromised patients in hospitals. Some vitamins are partially destroyed, such as vitamin C, and it would be unwise to eat only irradiated fruit and vegetables. The main cause for concern is that it may be used by unscrupulous traders to 'clean up' consignment food, particularly shellfish, with high bacteria counts. Bacterial toxins would remain in the food, so that it could still cause illness, eventhough irradiation would have removed signs of live bacteria (Sommers and Fan, 2008). Stringent regulations would be needed to prevent this from happening. Other damaging changes may take place in the food, such as the creation of free radicals, but research so far suggests that the process is relatively safe (Peter and Julian, 1999).

2.3.11 Puffing

It is a method of processing cereal grains. They are subjected to high pressures, and then suddenly ejected into a normal atmospheric pressure, causing the grain to expand sharply (Doona and Feeherry, 2008). This is used to make puffed wheat cereals and puffed rice cakes (Peter and Julian, 1999).

2.3.12 Chemical Treatments

The techniques are widely used, for example in manufacturing margarine, in which hydrogen is bubbled through vegetable oils in the presence of a catalyst to produce more solid, spreadable fat. The catalyst is later removed. Chemicals introduced in processing that remain in the food are known as food additives and include flavorings, preservative, anti-oxidants, emulsifiers, and colorings (Peter and Julian, 1999). All in all, the summarization types of preservation technology are illustrated in Table 2.1. The applications, advantages and disadvantages are listed in the table.

Table 2.1: Summarization types of preservation technology

PRESERVATIONS TECHNOLOGY			
TYPES	**APPLICATIONS**	**ADVANTAGES**	**DISADVANTAGES**
Refrigeration	Below 5°C/41°F (or below 3°C/37°F for cooked foods) slows the processes of spoilage	Effective for foods with low water content.	• Cannot kill microorganisms, nor stop their growth completely • Limitations cause many case of food poisoning • Less effective for foods with high water content • Refrigerator temperatures should be checked as the efficiency of the machinery could decline with age and higher temperature is dangerous
Deep Freezing	Deep freezing (-18°C or below) stops almost all spoilage processes, except residual enzyme activity in uncooked vegetables and most fruits, which are blanched (dipped in hot water to destroy the enzymes) before freezing.	• Unavailable to microorganism by converting it to ice. • Microorganisms cannot grow or divide while frozen.	• Most microorganisms remain alive and can resume activity once defrosted. Some foods are damaged by freezing, notably soft fruits and salads, the cells of which are punctured by ice crystals, leading to loss of crispness. • Fatty foods such as cow's milk and cream tend to separate.
Pasteurization	It is used mainly for milk.	Disease-causing bacteria can be destroyed	Less harmful bacteria survive, so the milk will still go sour within a few days

Ultra-heat Treatment	It is used to produce UHT milk.	Kills all bacteria present, giving the milk a long shelf life	Altering the flavor
Drying	Products such as fruit, dried milk and instant coffee.	• It is effective because both microorganisms and enzymes need water to be active. • Drying concentrates the soluble ingredients in foods (prevents the growth of bacteria, yeasts and moulds).	Dried food will deteriorate rapidly if allowed to become moist.
Freeze-drying	• Quality instant coffee and dried vegetables. • This method of preservation is commercially used but the products are most often used as constituents of composites dishes, such as packet meals. • Fruit, vegetables, meat, and fish have proved satisfactory.	• It is less damaging to food than straight dehydration in the sense that foods reconstitute better. • Retain the original size and shape. They have a sponge like texture, and rapidly reabsorb liquid when reconstituted (most acceptable for thin pieces)	• The foods lose much of their weight. • Refrigeration is unnecessary during storage; the shelf life is similar to dried foods, provided the product is not allowed to become moist. • The method is not recommended for pieces thicker than 3cm.
Canning	Drinks are canned to preserve the carbon dioxide that make them fizzy	• The food is sealed in a can to prevent contamination.	The effect of heat processing on the nutritive value of food is variable.
Pickling	To make yoghurt and cheese	Stops the growth of moulds.	

Curing of meat	It involves soaking in salt (sodium chloride) solution, with saltpeter (sodium nitrate) added to give the meat its pink tinge and characteristic taste.		Bacteria convert the nitrates in cured meats to nitrites and nitrosamines, which are potentially carcinogenic to humans
Irradiation	• It is a method of preserving food by subjecting it to low-level radiation. Irraddiated food is used for astronauts and for immunocompromised patients in hospitals.	The exposure of food to low-level irradiation is to kill microorganisms. Irradiation is highly effective, and does not make the food any more radioactive than it is naturally.	• Bacterial toxins would remain in the food, so that it could still cause illness, even though irradiation would have removed signs of live bacteria. • Other damaging changes may take place in the food, such as the creation of free radicals

2.4 FOOD PRESERVATION USING FREEZING MECHANISM

Freezing is a method of food preservation whereby the heat is removed (heat of fusion), temperature of the food is reduced below its freezing point ($T<T_f$) and a portion of water in food undergoes a change in state to form ice crystals (A_w lowered). Preservation by freezing achieved by the low temperature, reduced water activity due to ice formation and high concentration of solutes in unfrozen water and also by blanching some foods (Barbosa-Canovas et al., 2008). The goal of freezing is to prevent the growth of microorganisms which is by stabbing some bacteria (little effect), reducing water activity, formatting mechanism of ice crystals, changing of the osmotic in cell fluids, tying up some free water and lowering the temperature enough to slow down chemical reactions at every 10°C decrease in temperature halves the reaction rate (Juan and Stojanovic, 2005).

2.4.1 Freezing Theory

A general definition of freezing is energy which is ability to work and heat energy in transit (dynamic) due to temperature difference between the source and the products. Specific heat in freezing is the quantity of heat that is gained or lost by a unit mass of product to accomplish a unit change in temperature without the change in state (kJ/kg C). While sensible heat is the heat when added or subtracted from material changes their temperature and the changes can be sensed. The latent heat during freezing is the heat required to change the physical state of materials at constant temperature. The basis for freezing foods is water (Tewari and Juneja, 2008). The Figure 2.1 indicates the water content of various foods categories.

Food	Water content(%)	Freezing Point (°C)
Vegetables	78-92	-0.8 to -2.8
Fruits	87-95	-0.9 to -2.7
Meat	55-70	-1.7 to -2.2
Fish	65-81	-0.6 to -2.0
Milk	87	-0.5
Egg	74	-0.5

Figure 2.1: Water content (%) for various types of foods

Source: Juan and Stojanovic (2005)

When water is chilled it reaches its max density at 4°C (Sg= 1) and freezes at 0°C (Sg= 0.917). That is the cause of ice floating in water (Juan and Stojanovic, 2005).

2.4.2 Food Composition

Foods are made of water and chemicals. Foods contain protein, fats, carbohydrates, minerals, vitamins, and enzymes. The water may be free off or bound to other components in food. All water in foods does not freeze. Frozen water is at -20°C. Examples of food which contains water include lamb (88%), fish (91%) and egg (93%). Although food mostly consists of water, it contains a lot of soluble materials. Soluble materials slow down the movement of water molecules, and the freezing occurs at a lower temperature. In addition, 1gmol of soluble matter will decrease (lower) the freezing point by 1.86°F (1°C). The examples of freezing points for fruits and vegetables are at 29°F to 30°F while meat and fish at 27°F to 28°F (Juan and Stojanovic, 2005).

2.4.3 Freezing Curve

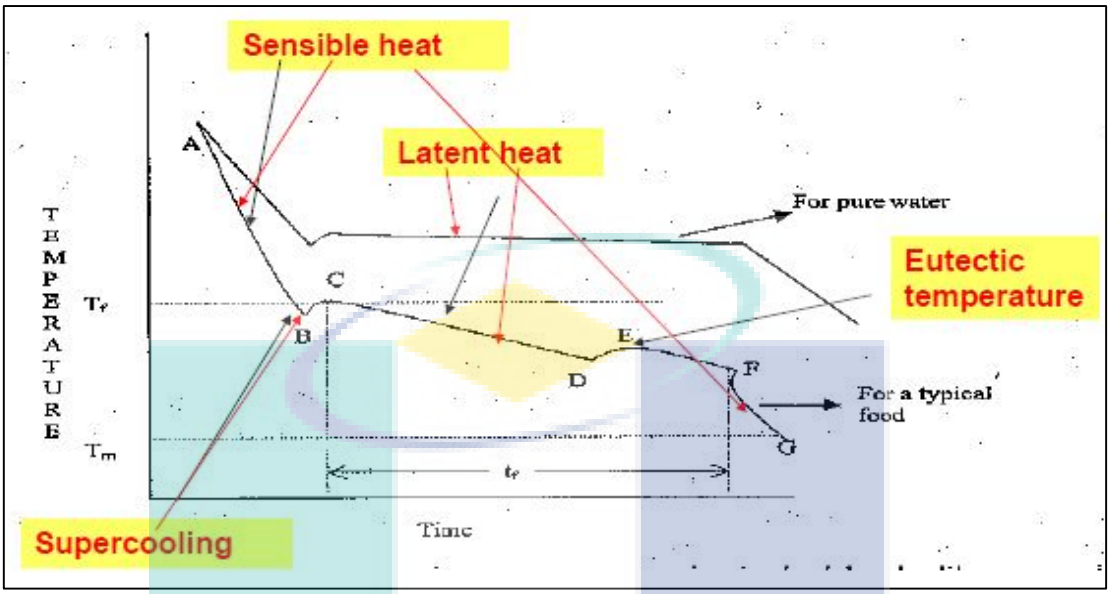

Figure 2.2: Freezing curve for pure water and typical food

Source: Juan and Stojanovic (2005)

Figure 2.2 above illustrates the freezing curve for pure water and typical food. At point AB, food undergoes cooling at below freezing point (less than 0) and at point B water remains liquid although the temperature is below 0°C.This phenomenon is called supercooling. Supercooling is a process in which food goes below freezing point without the formation of ice crystals (crystallization). It yields better quality food if water is not present. This shows that the undesirable effects of freezing are due to ice formation rather than the reduction of temperature. While at point BC, the temperature rises rapidly to the freezing point (giving off heat of fusion) and ice crystals begin to form. The latent heat of crystallization is released at this time. Furthermore, at point CD, heat is removed as latent heat so the temperature remains constant. A major part of ice is formed and in unfrozen liquid there is an increase in solute concentration and that is the reason that brings the temperature to fall slightly. Next, at point DE one of the solutes becomes supersaturated and crystallizes out. The latent heat of crystallization is realized and the temperature rises to eutectic point for that solute. Eutectic point is where the temperature has no further concentration of solutes due to freezing, thus the solution freezes. The temperature at which a crystal of individual solute exists in

equilibrium with the unfrozen liquor and ice thus it is difficult to determine individual eutectic points in the complex mixtures of solutes in foods. Hence term final eutectic point is used. The lowest eutectic temperature of the solutes was found in the food. Examples of eutectic temperature in food are ice cream which is at -55^{o}C, meat (-50^{o}C to -60^{o}C) and bread (-70^{o}C). Maximum ice crystals formation is not possible until this temperature is reached. For point FG, the temperature of the ice water mixture falls to the temperature of the freezer and percentage of water remains unfrozen. Food frozen below point E forms a glass which encompasses the ice crystals (Juan and Stojanovic, 2005).

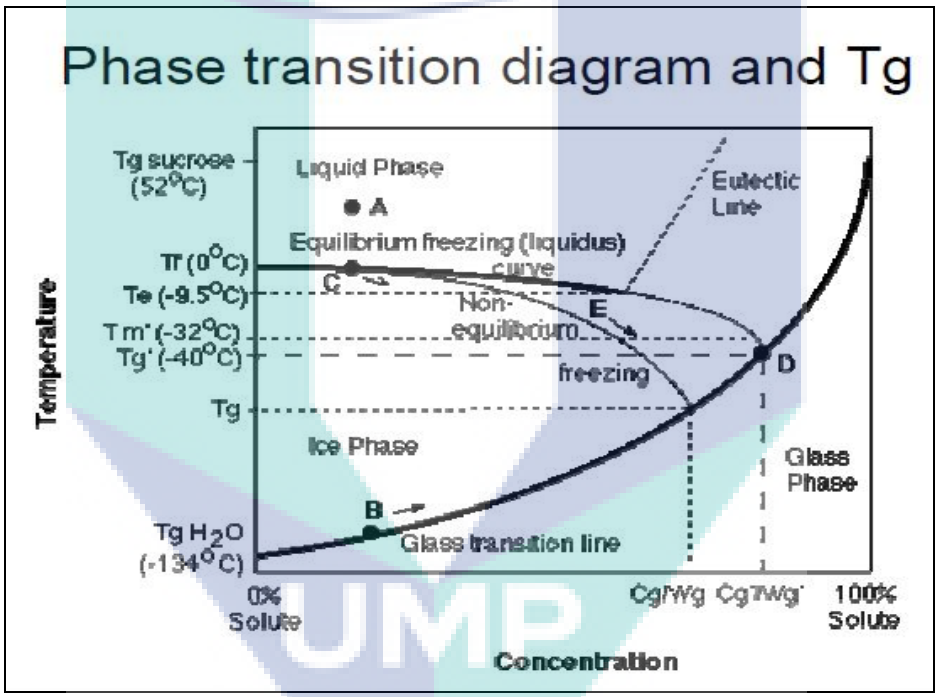

Figure 2.3: The illustrative phase transition diagram and glass temperature (Tg)

Source: Juan and Stojanovic (2005)

An illustrative phase transition diagram and the glass temperature point are shown in Figure 2.3. Glass transition temperature is the temperature at which the products undergo a transition from rubbery to glassy state. Formation of glass protects the texture of the foods and gives good storage stability (Juan and Stojanovic, 2005).

2.4.4 Ice Crystal

Ice crystals consist of nucleation (site for crystal formation and growth). An association of molecules into a tiny ordered particles sufficient to survive and serve as a site for crystal growth. It can be homogenous (pure water), heterogeneous (most foods) and dynamic (spontaneous). The crystal growth (where it is formed) is the enlargement of the nucleus by the orderly addition of molecules. Crystal growth can occur at temperatures just below melting point while nucleation starts at a lower temperature (supercooling). Heat transfer is the most responsible for limiting the rate of crystallization due to the large amount of latent heat needed (Juan and Stojanovic, 2005).

The location of ice crystals in tissue is the function of freezing rate (slow and rapid), specimen temperature and nature of the cell. Intercellular ice crystal grows when water vapours move from inside cells to cellular space because of vapour pressure differences. Rate and size are governed by the freezing rate applied to the products (Mousavi et al., 2005). Slow freezing will lead to large inter cellular ice crystal and fast freezing will cause to small inter cellular of ice crystal (Juan and Stojanovic, 2005). The crucial impact of freezing rate on the end product quality is shown in Figure 2.4 below.

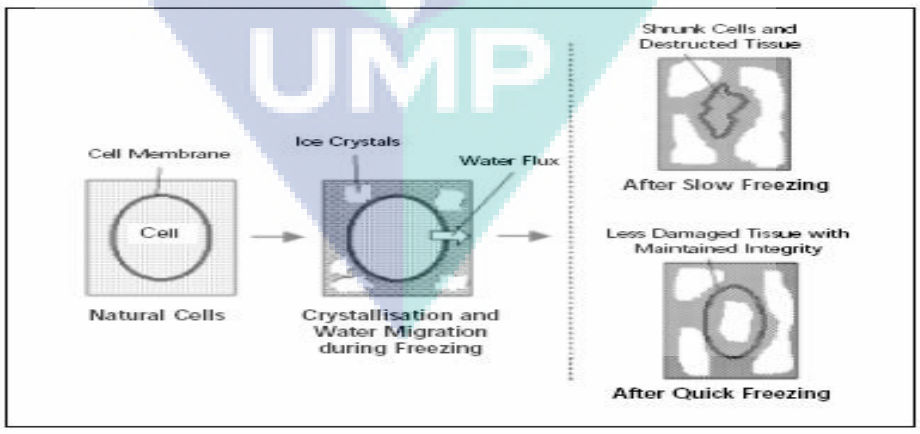

Figure 2.4: The crucial impact of freezing rate on the end product quality

Source: Juan and Stojanovic (2005)

2.4.5 Types of Freezing Rate

There are two types of freezing; slow and rapid freezing rate. Slow freezing is when the rates of cooling were less than 1°C/min. The ice crystals form in extracellular locations and slow freezing will lead to large ice crystals formation, maximum dislocation of water, and shrinkage (shrunk appearance of cells in frozen state) but less than maximum attainable food quality. Rapid freezing will produce both extracellular and intracellular (mostly) locations of ice crystals. This freezing technique will lead to small and numerous ice crystals and minimum dislocation of ice crystals (Mousavi et al., 2005). Rapid freezing preserves most of its frozen appearance similar to the unfrozen state and the food quality is usually superior to that attained by slow freezing (Juan and Stojanovic, 2005). The Figure 2.5 below shows rapid versus slow freezing rate mechanism.

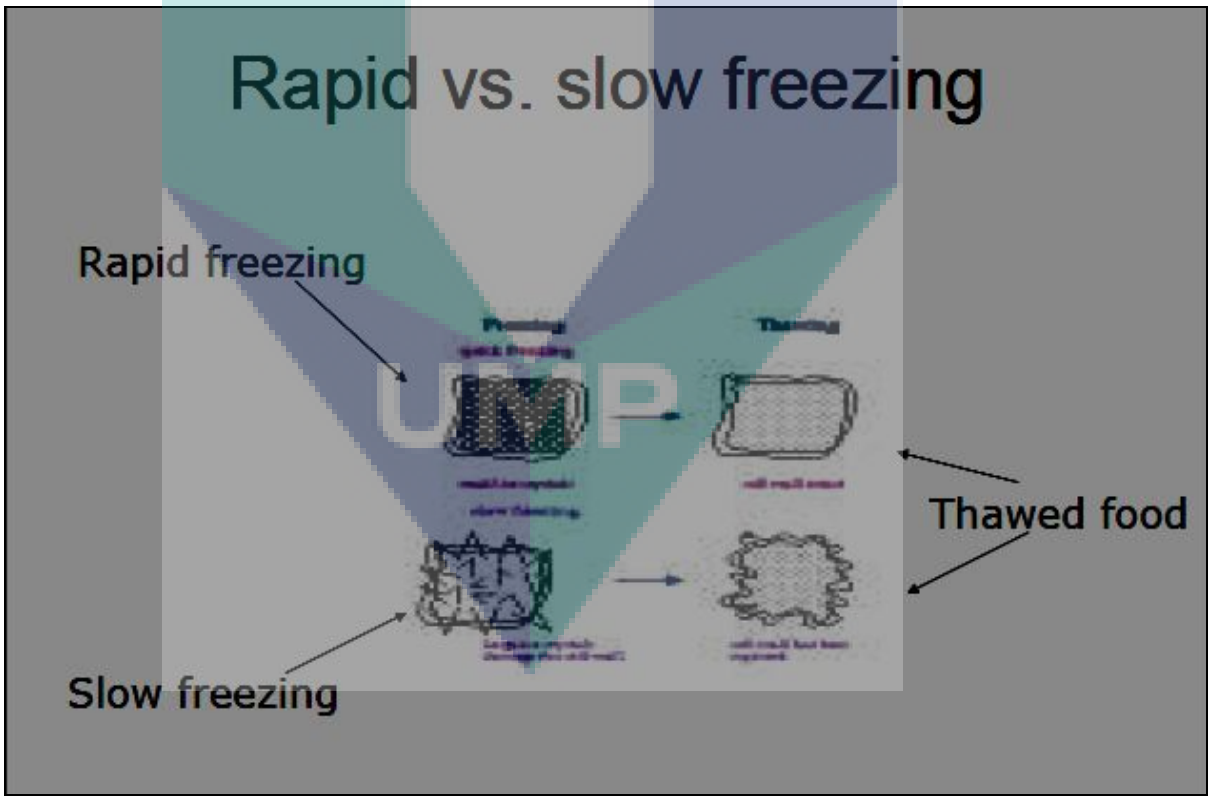

Figure 2.5: Mechanism of rapid versus slow freezing rate

Source: Juan and Stojanovic (2005)

2.4.6 Chemical and Physical Affect of Freezing

The volume of ice is 9% greater than the volume of water after the freezing process takes place. Expansion of foods after freezing would be expected and depended on moisture content, cell arrangement, concentration of solutes (higher concentration less expansion) and freezer temperature. Chemical effects of freezing are concentration changes of chemicals in liquid phase. It will increase acidity and lower pH (protein denaturation) and these effects are more pronounced during storage and slow freezing (Juan and Stojanovic, 2005). Types of chemical changes are:

i) flavor and odor deterioration,
ii) pigment degradation,
iii) enzymatic browning,
iv) autoxidation of ascorbic acid,
v) protein insolubilization, and
vi) lipid oxidation.

There are few factors that affect chemical changes such as initial substrate concentration, pH level, water activity, oxygen, handling and processing and also time and temperature. Prevention of chemical changes can be done as follows:

i) inactivation of enzymes,
ii) low temperature storage,
iii) alternation of pH, and
iv) exclusion of oxygen.

2.4.7 Microbiology of Frozen Food

Growth of microorganisms is temperature dependent. No pathogens can grow around 5ºC and there is no microorganisms' growth at less than -5 ºC. Freezing cannot kill pathogens if food is already contaminated. However some microorganisms are killed and injured but some are found still alive. Microorganism during freezing and storage does not cause any problem but after thawing if it is controlled then there will also be of no problem. However if it is uncontrolled then only it will create food safety

issues (Juan and Stojanovic, 2005). For example, the number of bugs surviving freezing depends on:

i) number of bugs,
ii) type of bug,
iii) storage temperature,
iv) method of measurement, and
v) temperature fluctuations (decrease).

In order to solve these problems, the process is needed to control the initial load, freeze it rapidly, store at -18^{o}C (constant), thaw rapidly (low temperature), or use it immediately or cook and store it at about 5^{o}C.

2.4.8 Factors that Affect Freezing (rate)

Factors that affect freezing (rate) are (Mousavi et al., 2005):

i) characteristics of the food in terms of composition,
ii) thermal conductivity (Absolute refrigeration required ,i.e. ability of system to remove heat),
iii) temperature difference (initial product temperature; between product and medium),
iv) food size (volume to area, product thickness), and
v) insulating effects (air velocity, package-contact between product and medium)

2.4.9 Freezing Types (Equipments)

There are few types of freezing equipment which includes (Juan and Stojanovic, 2005):

i) mechanical (direct and indirect),
ii) cryogenic (CO_2, N_2),
iii) slow freezers and sharp freezers (0.2 cm/h) including still-air freezers and cold stores,
iv) quick freezers (0.5-3 cm/h) including air-blast and plate freezers,
v) rapid freezers (5-10 cm/h) including fluidized-bed freezing, and

vi) ultra-rapid freezers (10-100 cm/h), that is cryogenic freezers

Examples of freezing equipment are as followed:

i) Chest and sharp freezers:

Chest and sharp freezers (Figure 2.6) are inexpensive and the temperature for these types of freezing equipment is about -20^{o}C and -30^{o}C. It is a slow freezing mechanism and there is no mechanical movement involved during freezing process (Juan and Stojanovic, 2005).

Figure 2.6: Chest and sharp freezers

ii) Blast freezers:

Temperatures for these types of freezing equipment is around -30^{o}C and -40^{o}C at a velocity of 1.5 to 6.00 ms^{-1}. Many configurations are possible. It is a fast freezing rate equipment and applicable for continuous and batch process (Juan and Stojanovic, 2005). However there is a potential concern in which freezer can burn and cause dehydration (counter current flow).

iii) Ice-cream or scraped-surface freezer:

Ice cream or scraped-surface freezer (Figure 2.7) is used for pre-freezing of fluid products (ice-cream mix). This equipment finished freezing in the “hardening room” (Juan and Stojanovic, 2005).

Figure 2.7: Ice-cream or scraped surface freezer

iv) Belt or spiral freezers:

There are modified air-blast freezers in which a continuous flexible mesh belt is formed into spiral tiers. Spiral freezers require relatively small floor-space and have high capacity (for example a 50-75 cm belt in a 32-tier spiral processes up to 3000kgh-1). Other advantages include automatic loading and unloading, low maintenance costs and possess flexibility for different products. They are used for a wide range of foods including pizzas, cakes, pies, ice cream, whole fish and chicken portions. Figure 2.8 below shows the inner components of the belt or spiral freezer equipment (Juan and Stojanovic, 2005).

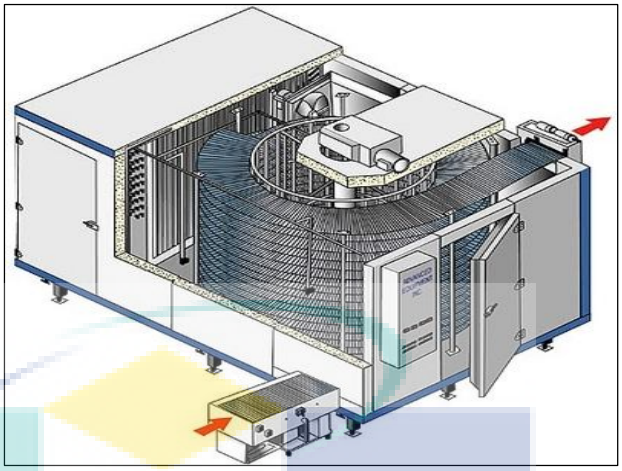

Figure 2.8: Inner part of belt or spiral freezers

v) Fluidized bed freezer:

Fluidized bed freezer (Figure 2.9) has high air flow velocities: 2-5 m/s with a bed depth around 2-13 cm and both are determined by food size and shape. It has higher heat transfer coefficients, shorter freezing times, higher production rates (l0, 000kgh-1) and less dehydration of unpackaged food than blast freezing. Obviously it is limited only to particulate foods (Juan and Stojanovic, 2005).

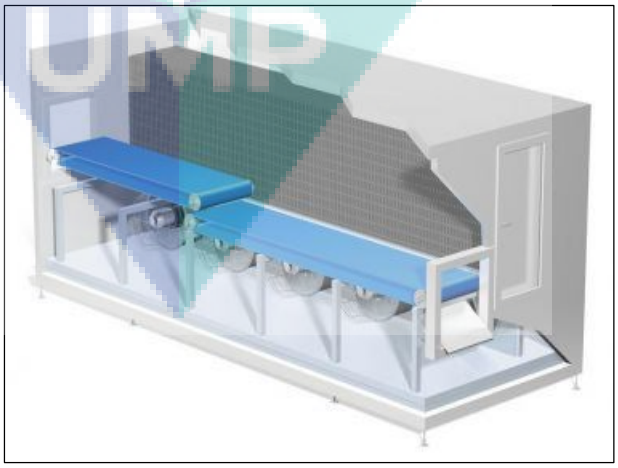

Figure 2.9: Fluidized Bed freezer

vi) Plate freezer:

Advantages of plate freezer equipment (Figure 2.10) are good use of floor space, low operating costs, less dehydration of food, high rates of heat transfer, and unchanged food package dimensions. However the disadvantages of this equipment are very high capital cost and the size limitations (Juan and Stojanovic, 2005).

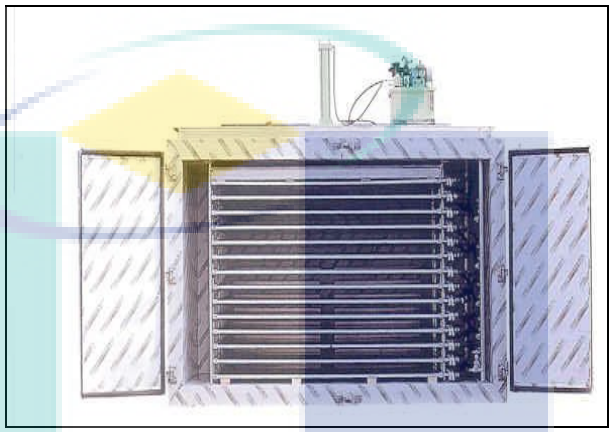

Figure 2.10: Plate Freezer

vii) Cryogenic freezers:

Cryogen may be sprayed on food or food may be immersed in cryogen. Most common refrigerants are not fluorocarbons. There are two types of common cryogenic freezers. Liquid nitrogen is one of the cryogen that had been used in which 48% of the total freezing capacity (enthalpy) is taken up by the latent heat of vaporization needed to form the gas; 52% of the enthalpy is available in the cold gas. Other than that the carbon dioxide is another cryogen and its freezing capacity (85%) is available from the subliming solid (Juan and Stojanovic, 2005). The properties of foods cryogen is illustrated in Figure 2.11.

	Liquid N_2	CO_2
Density (kg m^{-3})	784	464
Specific heat (liquid) (kJ $kg^{-1}K^{-1}$)	1.0	2.2
Latent heat (kJ kg^{-1})	358	352
Total usable refrig. effect (kJ kg^{-1})	690	565
Boiling point (C)	-196	-78.5 (sub)
Consumption per 100 kg of product frozen (kg)	100-300	120-375

Figure 2.11: Properties of food cryogens

Source: Juan and Stojanovic (2005)

Selection of freezer equipment depends on the cost of that equipment, rate of freezing required, size, shape, package and also either a batch or a continuous process. A comparison of freezing methods is illustrated in Figure 2.12.

Method of freezing	Typical film heat transfer coefficient	Typical freezing times for specified foods to -18C (min)	Food
Still air	6-9	180-4320	Meat carcass
Blast (5 ms^{-1})	25-30	15-20	Unpackaged peas
Blast (3 ms^{-1})	18		--
Spiral belt	25	12-19	Hamburgers; fish fingers
Fluidized bed	90-140	3-4	Unpacked peas
		15	Fish fingers
Plate	100	75	25 kg blocks of fish
		25	1 kg carton vegetables
Scraped surface	--	0.3-0.5	Ice cream (layer ca.1mm thick)
Cryogenic (liquid N)		1.5	454 g of bread
	1500	0.9	454 g of cake
		2-5	Hamburgers; seafood
		.5-0.6	Fruits and vegetables

Figure 2.12: Comparison of freezing methods

Source: Singh and Heldman (1993)

2.4.10 Frozen Food Quality Issues

The key factors in frozen food quality issues are its storage temperature in which generally is the colder the better but this will be very costly and cause temperature fluctuations. Practical Storage Life (PSL) Time that the product maintains its sensory

quality or functionality is another factor in frozen food quality issues. High Quality Life (HQL) issues which is time from freezing until a statistically significant change in quality, is perceived by a sensory panel. Just Noticeable Difference – (JND) is the practical storage life of frozen foods as influenced by storage temperature is also another matter (Singh and Heldman, 1993). Frozen food quality occurred during:

i) pre-freezing,
ii) freezing,
iii) packaging,
iv) storing,
v) distributing/transporting,
vi) retailing, and
vii) thawing (consumer)

As an example, the loss in quality of strawberries during a typical manufacturing through distribution chain is given by Singh and Heldman (1993) and it is summarized in Figure 2.13.

Stage	Time (Days)	Temperature (C)	Acceptability (Days)	Loss per day (%)	Loss (%)
Producer	250	-22	660	0.15152	37.88
Transport	2	-14	220	0.45455	0.91
Wholesale	50	-23	710	0.14085	7.04
Transport	1	-12	140	0.71429	0.71
Retail	21	-11	110	0.90909	19.09
Transport	0.1	-3	18	5.55556	0.56
Home freezer	20	-13	180	0.55556	11.11
Total storage (days)	344.1			Total quality loss	(percent) = 77.30

Figure 2.13: The loss in the quality of strawberries during a typical manufacturing through distribution chain

Source: Singh and Heldman (1993)

2.5 FOOD PRESERVATION USING DRYING MECHANISM

Drying or dehydration involves the removal of water from the food by controlled processes. This can be done by evaporation due to heating of the product (drying of fruits), osmotic dehydration (brining of fish) and sublimation, or freeze drying (drying of coffee). There are two distinct stages in this technology. In the first stage, the removal of surface water depends solely on the state of the air surrounding the food; such as its temperature, relative humidity and speed. In the second phase of drying, the moisture within the food moves to the surface. As the air is heated, its relative humidity decreases, resulting in more absorption of water. Here the rate of drying is dependent on the time the moisture takes to get to the surface. The heating of the air around a food product can therefore cause it to dry more quickly. By the same token, as the food loses moisture, the time the moisture takes to get to the surface becomes increasingly longer and drying therefore becomes slower as shown in Figure 2.14. It should be noted however, that although the shortest drying time may be preferred, in the case of starchy foods, the common change of "case hardening" may result. In such an event, water removal from the surface is much faster than the rate at which water migrates from the interior. The surface, therefore, dries into a hard layer, which actually prevents the migrating water from reaching the surface (Audrey et al., 2004).

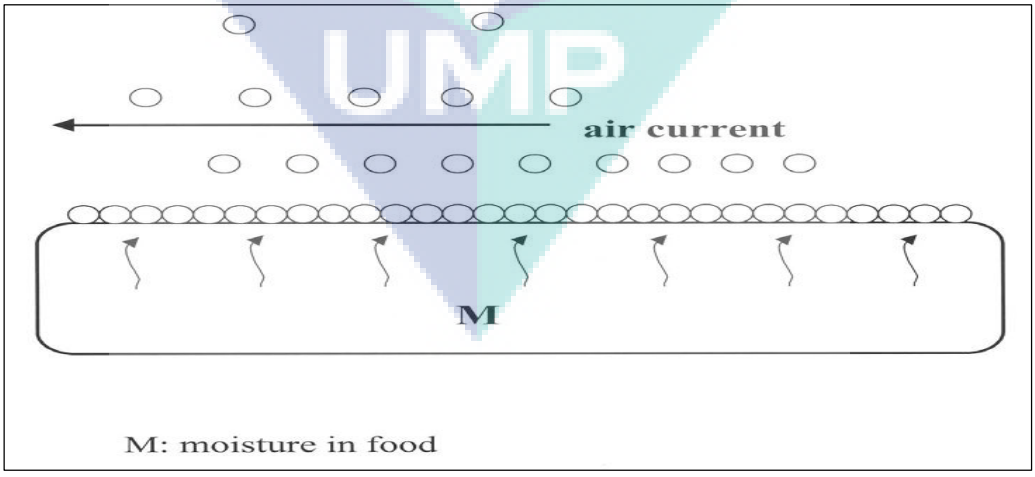

Figure 2.14: Removal of water vapour

Source: Audrey (2004)

Advantages of drying process to preserved foods are as followed (Audrey et al., 2004):

i) Long Shelf Life – since most microorganisms are responsibled for food spoilage are unable to grow and multiply in the absence of moisture, spoilage due to microbial degradation is limited in dried foods. Furthermore, enzymes which catalyse undesirable changes in foods need moisture to be effective,

ii) Reduced Weight – this result in reduced transportation, storage and shipping costs,

iii) Convenience – The production of convenience items with novelty appeal for niche markets makes drying an attractive option,

iv) Concentration of nutrients – The removal of most of the water from a food results in a highly concentrated source of nutrients, and

v) No refrigeration is required for dried products – This would save on energy and storage costs. Together with a long shelf life would provide a lucrative processing alternative for tropical countries.

Disadvantages of drying are few, and mainly it is related to oxidation, which usually accompanies drying. This results in losses of micronutrients such as carotene and ascorbic acid, and minimal loss in protein as a result of browning reactions. Reduced consumer appeal is often linked with the latter. There might also be changes in flavour and texture if drying is not properly controlled, particularly in regards to maximum temperatures (Audrey et al., 2004).

Drying of foods is particularly important for the handling and distribution of raw materials with high moisture content and a limited shelf-life such as fruits and vegetables. A number of attempts have been made to establish novel drying techniques for improving not only product quality, but also economic efficiency (Bohm et al., 2006; Sankat et al., 1996; Yongsawatdigul and Gunasekaran, 1996; Venkatachalapathy and Raghavan, 1999, and Erle and Schubert, 2001).

The term drying refers generally to the removal of moisture from a substance. It is the most common and most energy-consuming food preservation process. With literally hundreds of variants actually used in drying of particulate solids, pastes,

continuous sheets, slurries or solutions, it provides the most diversity among food engineering unit operations (Ratti, 2001, and Ratti and Mujumdar, 1995).

2.5.1 Types of Drying

Most flavours are retained in this method of preservation; the product is less bulky (reduced shipping costs) and the shelf life is extended. Drying is an excellent way of preserving several Caribbean's seasonal fruits during the off season. There are several types of dryers which are used (Audrey et al., 2004). These include:

i) drum dryer,
ii) cabinet dryer,
iii) tunnel dryer,
iv) drum/rotary dryer,
v) spray dryer, and
vi) solar dryer

2.5.2 Basic Method of Drying Mechanism

2.5.2.1 Air and Contact Drying under Atmospheric Pressure

In this case, the heat is transferred through the food either from heated air or heated surfaces, and the resulting water vapour is removed with the air current (Figure 2.14). Solar, sun, drum and spray drying utilize this technique (Audrey et al., 2006).

2.5.2.2 Vacuum Drying

Since evaporation of water takes place more readily at lower pressures, drying under vacuum is faster. This method is more expensive than air-drying and is reserved for specialised products (Audrey et al., 2004).

2.5.2.3 Freeze-drying

Water is removed by sublimation from frozen foods. The food structure is better conserved by using this equipment even though the maintenance is costly (Audrey et al., 2004).

2.5.2.4 Solar Drying or Sun Drying

Sun drying and solar drying are obvious alternatives for this region due to the abundance of natural sunlight. Although the two terms are sometimes used interchangeably, for the purpose of this manual, sun drying refers to the removal of moisture by merely placing the commodity in the sun (barbecue, and rack). Limitations of traditional sun drying include the following (Audrey et al., 2004):

i) moisture loss is intermittent, as it is largely dependent on the weather,
ii) drying rates are usually slow and do not result in high quality products,
iii) moisture levels are too high for prolonged storage, and
iv) insect infestation.

Solar drying, however, involves capturing and concentrating solar energy for the purpose of removing water. This method has increased in popularity, although commercial solar dryers with high rates of efficiency are often quite expensive. The advantages of solar drying over sun drying include (Audrey et al., 2004):

i) faster drying rates as higher air temperatures are generated,
ii) lower final moisture content of the finished product,
iii) greater protection of the product from rain, dust, and pests, and
iv) low insect and mould infestation due to higher temperatures.

Essentially, there are two types of solar dryers – direct and indirect. Regardless of which type is used, it is important to have information on the seasonal and daily variation of sunshine, humidity, temperature, wind speed and wind direction during drying. When the direct solar dryer is used, air is heated in the drying chamber, which acts both as the solar collector and dryer. The indirect dryer on the other hand comprises two parts – a solar collector, and a drying chamber for the crop. Air enters the collector

where it is heated and its humidity decreases (Audrey et al., 2004). When selecting a solar dryer, consideration of the following factors aid in assuring success:

i) The mixed mode dryer maximises the utilisation factor of the capital investment. Design may allow the dryer to be used for heating water for domestic purposes.

ii) An additional heat source is recommended to ensure continuous drying even when there is no sunshine, also to handle peak loads and improve efficiency. Forced convection indirect dryers have the advantages of providing better control, more uniform drying and smaller collector areas.

iii) Small dryers allow for diversity in terms of crops dried. However, due to the high fresh weight to dry weight ratio, the dryer should be large enough to provide feasible throughput. Figure 2.15 shows a simple solar dryer.

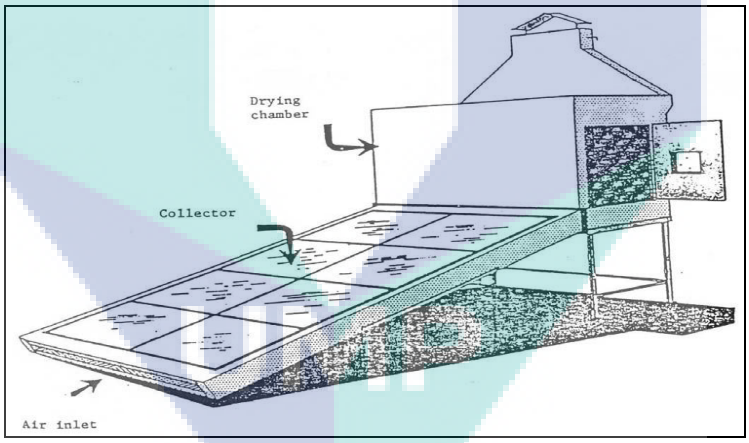

Figure 2.15: Simple solar dryer

2.6 FREEZE-DRYING

Freeze-drying (also known as lyophilization or cryodesiccation) is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport. Freeze-drying works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to the gas phase. Freeze drying is a

relatively recent method of preserving food. It involves freezing the food, then removing almost all the moisture in a vacuum chamber, and finally sealing the food in an airtight container (Hui et al., 1992). Freeze dried foods can be easily transported at normal temperatures, stored for a long period of time, and consumed with minimum preparation. Once prepared, the freeze-dried foods have much the same look and taste as the original, natural products.

The freeze-drying process was developed during World War II as a method of preserving blood plasma for battlefield emergencies without requiring refrigeration or damaging the organic nature of the plasma (Hui et al., 1992). The technology was applied to consumer food products after the end of the war. Coffee was one of the first freeze-dried products to be marketed on a large scale. Today, many fruits, vegetables, meats, eggs, and food flavourings are freeze-dried.

Freeze-dried food has many advantages. Because of as much as 98% of the water content has been removed, the food is extremely lightweight; this significantly reduces the cost of shipping. This also makes it popular with boaters and hikers who have to carry their food with them. Because it requires no refrigeration, shipping and storage costs are even further reduced. Freeze-dried food is also relatively contamination-free since the dehydration process makes it virtually impossible for yeast and potentially harmful bacteria to survive (Hui et al., 1992). Finally, since the physical structure of the food is not altered during the freeze-drying process, the food retains much of its colour, shape, texture, and flavour when it is prepared for consumption by reintroducing water (Hui et al., 1992). This makes it more attractive to consumers than food preserved by some other methods.

2.6.1 Freeze Drying Process

There are three stages in the complete drying process: freezing, primary drying, and secondary drying.

2.6.1.1 Freezing

In a lab, this is often done by placing the material in a freeze-drying flask and rotating the flask in a bath, called a shell freezer, which is cooled by mechanical refrigeration, dry ice and methanol, or liquid nitrogen. On a larger-scale, freezing is usually done using a freeze-drying machine. In this step, it is important to cool the material below its triple point, the lowest temperature at which the solid and liquid phases of the material can coexist. This ensures that sublimation rather than melting will occur in the following steps. Larger crystals are easier to freeze-dry. To produce larger crystals, the product should be frozen slowly or can be cycled up and down in temperature. This cycling process is called annealing. However, in the case of food, or objects with formerly-living cells, large ice crystals will break the cell walls (discovered by Clarence Birdseye), resulting in cell destruction, and, in the case of rehydrated foods, result in a poor texture. In this case, freezing is done rapidly, in order to lower the material to below its eutectic point quickly, thus avoiding the formation of ice crystals. Usually, the freezing temperatures are between −50 °C and −80 °C. The freezing phase is the most critical in the whole freeze-drying process, because the product can spoil if it is not properly done. Amorphous materials do not have a eutectic point, but do have a critical point, below which the product must be maintained to prevent melt-back or collapse during primary and secondary drying.

2.6.1.2 Primary Drying

During the primary drying phase, the pressure is lowered (to the range of a few millibars), and enough heat is supplied to the material for the water to sublimate. The amount of heat necessary can be calculated using the sublimating molecules' latent heat of sublimation. In this initial drying phase, about 95% of the water in the material is sublimated. This phase may be slow (can be several days in the industry), because, if too much heat is added, the material's structure could be altered.

In this phase, pressure is controlled through the application of partial vacuum. The vacuum speeds sublimation, making it useful as a deliberate drying process. Furthermore, a cold condenser chamber and/or condenser plates provide a surface(s) for

the water vapour to re-solidify on. This condenser plays no role in keeping the material frozen; rather, it prevents water vapor from reaching the vacuum pump, which could degrade the pump's performance. Condenser temperatures are typically below −50 °C (−60 °F). It is important to note that, in this range of pressure, the heat is brought mainly by conduction or radiation; the convection effect is considered to be inefficient.

2.6.1.3 Secondary Drying

The secondary drying phase aims to remove unfrozen water molecules, since the ice was removed in the primary drying phase. This part of the freeze-drying process is governed by the material's adsorption isotherms. In this phase, the temperature is raised higher than in the primary drying phase, and can even be above 0 °C, to break any physico-chemical interactions that have formed between the water molecules and the frozen material. Usually the pressure is also lowered in this stage to encourage desorption (typically in the range of microbars, or fractions of a pascal). However, there are products that benefit from increased pressure as well. After the freeze-drying process is complete, the vacuum is usually broken with an inert gas, such as nitrogen, before the material is sealed. At the end of the operation, the final residual water content in the product is extremely low, around 1% to 4%.

There are many quality factors to consider during freeze-drying. Some of them include rehydration, color, texture, appearance, and freeze-drying time. It was found the most important factors during the freeze-drying process to be working pressure and heating plate temperature. The optimal conditions for freeze-drying strawberries was found at 30 Pa working pressure and 50°C heating plate temperature with the corresponding freeze-drying time between sixty and sixty-five hours (Harmit, 2005, and Hammami and Rene 1997).

There are many benefits to freeze-drying. First of all the product retains its initial properties such as shape, appearance, taste, color, flavor, texture, and biological activity (Hammami, 1997). Secondly freeze-dried products have a high rehydration capacity. These factors paired with the high yield and a longer shelf life make a freeze dried product superior to that of a fresh one. Furthermore, there is a reduction of weight for more convenient storage, shipping and handling. All in all, freeze drying makes a

highly perishable product extremely storable and easier to transport anywhere in the world (Hammami, 1997).

Although freeze-drying seems like a great process, there are also some drawbacks to the process. First of all, reconstitution can be an issue depending on the use of the freeze dried fruit. Research has found that the functional properties of the fruit can be altered as a result of different concentrations of reconstitution media (Harmit, 2005 and Mastrocola, 1997). This may be beneficial for producing formulated foods with a high added value. However, it may be detrimental when foods must be reconstituted for consumption. When using a reconstituted product for consumption, it is important to consider the time, process, type and concentration of the reconstitution media in which all have an effect on water uptake (Harmit, 2005).

Currently there are limited uses for freeze dried strawberries. Most of the freeze-dried processed fruit is used in cereals. Berry Burst Cheerios, Honey Bunches of Oats with Strawberries and Special K with red berries are some of these products. Freeze-dried strawberries are also sold as a novelty item labelled as "Astronaut Food" and as a dessert for backpackers or others planning to be out in the wilderness for lengthy periods. With the change in the food guide pyramid there is a potential increase for this type of food product. Snack bars which now use fruit flavoured pieces might include freeze-dried fruits as the demand for more fruits in the diet increases (Harmit, 2005).

2.6.2 Properties of Freeze-dried Products

Freeze-dried substance is sealed to prevent the re-absorption of moisture, the substance can be stored at room temperature without refrigeration, and be protected against spoilage for many years. Preservation is possible because the greatly reduced water content inhibits the action of microorganisms and enzymes that would normally spoil or degrade the substance. Freeze-drying also causes less damage to the substance than other dehydration methods using higher temperatures. Freeze-drying does not usually cause shrinkage or toughening of the material being dried. In addition, flavours, smells and nutritional content generally remain unchanged, making the process popular for preserving food (Packit, 2008). However, water is not the only chemical capable of

sublimation, and the loss of other volatile compounds such as acetic acid (vinegar) and alcohols can yield undesirable results. Freeze-dried products can be rehydrated (reconstituted) much more quickly and easily because the process leaves microscopic pores. The pores are created by the ice crystals that sublimate, leaving gaps or pores in their place. This is especially important when it comes to pharmaceutical uses. Freeze-drying can also be used to increase the shelf life of some pharmaceuticals for many years.

2.6.3 Applications of Freeze-drying

2.6.3.1 Pharmaceutical and Biotechnology

Pharmaceutical companies often use freeze-drying to increase the shelf life of products, such as vaccines and other injectables. By removing the water from the material and sealing the material in a vial, the material can be easily stored, shipped, and later reconstituted to its original form for injection.

2.6.3.2 Food Industry

Freeze-drying is used to preserve food and makes it very lightweight. The process has been popularized in the forms of freeze-dried ice cream, an example of astronaut food. It is also popular and convenient for hikers because the reduced weight allows them to carry more food and reconstitute it with available water. As examples:

i. Instant coffee is sometimes freeze-dried, despite high costs of freeze-dryers. The coffee is often dried by vaporization in a hot air flow, or by projection on hot metallic plates,
ii. Freeze-dried fruit is used in some breakfast cereal, and
iii. Culinary herbs are also freeze-dried, although air-dried herbs are far more common and less expensive. However, the freeze-drying process is used more commonly in the pharmaceutical industry.

2.6.3.3 Technological Industry

In chemical synthesis, products are often lyophilized to make them more stable, or easier to dissolve in water for subsequent use. In bio separations, freeze-drying can be used also as a late-stage purification procedure, because it can effectively remove solvents. Furthermore, it is capable of concentrating substances with low molecular weights that are too small to be removed by a filtration membrane. Freeze-drying is a relatively expensive process. The equipment is about three times as expensive as the equipment used for other separation processes, and the high energy demands lead to high energy costs. Furthermore, freeze-drying also has a long process time, because the addition of too much heat to the material can cause melting or structural deformations. Therefore, freeze-drying is often reserved for materials that are heat-sensitive, such as proteins, enzymes, microorganisms, and blood plasma. The low operating temperature of the process leads to minimal damage of these heat-sensitive products.

2.6.3.4 Other Uses

Organizations such as the Document Conservation Laboratory at the United States National Archives and Records Administration (NARA) have done studies on freeze-drying as a recovery method of water-damaged books and documents. While recovery is possible, restoration quality depends on the material of the documents. If a document is made of a variety of materials, which have different absorption properties, expansion will occur at a non-uniform rate, which could lead to deformations. Water can also cause moulds to grow or make inks bleed. In these cases, freeze-drying may not be an effective restoration method. As examples:

i. In bacteriology, freeze-drying is used to conserve special strain,
ii. In high-altitude environments, the low temperatures and pressures can sometimes produce natural mummies by a process of freeze-drying,
iii. Advanced ceramics processes sometimes use freeze-drying to create a formable powder from a sprayed slurry mist. Freeze-drying creates softer particles with a more homogeneous chemical composition than traditional hot spray drying, but it is also more expensive,

iv. Recently, few taxidermists have begun using freeze-drying to preserve animals, such as pets, and

v. Freeze drying is also used for floral preservation. Wedding bouquet preservation has become very popular with brides who want to preserve their wedding day flowers.

2.6.4 Freeze-drying Equipment

2.6.4.1 Rotary Evaporators

Rotary freeze-dryers are usually used with liquid products, such as pharmaceutical solutions and tissue extracts.

2.6.4.2 Manifold Freeze-dryers

Manifold freeze-dryers are usually used when drying a large amount of small containers and the product will be used in a short period of time. A manifold dryer will dry the product to less than 5% moisture content. Without heat, only primary drying (removal of the unbound water) can be achieved. A heater must be added for secondary drying, which will remove the bound water and will produce lower moisture content.

2.6.4.3 Tray Freeze-dryers

Tray freeze-dryers (Figure 2.16) are more sophisticated and are used to dry a variety of materials. A tray freeze-dryer is used to produce the driest product for long-term storage. A tray freeze-dryer allows the product to be frozen in place and performs both primary (unbound water removal) and secondary (bound water removal) freeze-drying, thus producing the driest possible end-product. Tray freeze-dryers can dry products in bulk or in vials. When drying in vials, the freeze-dryer is supplied with a stoppering mechanism that allows a stopper to be pressed into place, sealing the vial before it is exposed to the atmosphere. This is used for long-term storage, such as for vaccines.

Figure 2.16: Unloading trays of freeze-dried material from a small cabinet-type freeze-dryer

Improved freeze drying techniques are being developed to extend the range of products that can be freeze dried, to improve the quality of the product, and to produce the product faster with less labor. Since the 1930s, industrial freeze drying is depended on a single type of equipment: the tray freeze dryer. In 2005 a quicker and less-labor intensive freeze drying method is developed for bulk materials. This freeze drying process can produce free flowing powder from one single vessel. It is known as Active Freeze Drying (AFD) technology. The new process uses continuous motion to improve mass transfer and hence cutting processing time, while also eliminating the need to transfer to and from drying trays and downstream size reduction devices.

2.7 AIR-DRYING

Air-drying is the most conventionally used for dehydration process in the food and chemical industry which produces products that are characterized by low porosity and high apparent density (Brown et al., 2008, and Krokida and Maroulis, 1997). The high temperatures commonly used during industrial air-drying which is typically around 65^{o}C to 85^{o}C can cause damage to the microstructure and may also have a negative influence on the colour, texture, taste, aroma and nutritional value of the product thereby influencing the quality of both the dried and subsequently dehydrated product (Brown et al., 2008). Although there are several alternatives to air-drying such as microwave-, osmotic- and vacuum-drying, none is ideal in every respect (Vega-Mercado et al., 2001). For example, while microwave drying promotes quick drying,

difficulties in controlling the rapid mass transport may cause damage in the form of "puffing". Additionally, this technique is associated with high start-up costs and ongoing costs relating to the regular replacement of expensive magnetrons (Brown et al., 2008 and Nijhuis et al., 1998). Therefore, a place exists for new drying techniques that could be used in the food industry.

2.8 NUTRITIONAL AND QUALITY CHANGES DURING PROCESSING

A serving of fresh strawberries consist approximately 8 medium strawberries or approximately one half cup of fruit. Figure 2.17 depicts a nutritional label for 1 cup of fresh halved strawberries. As seen in the figure, fresh strawberries are packed with 3 grams of fibre and many antioxidants. Strawberries, with 149% Vitamin C, contain more of this vitamin than an orange. Furthermore, strawberries are packed with potassium, magnesium and calcium (Harmit, 2005).

Figure 2.17: Nutritional Label for Fresh Strawberries

Source: Harmit (2005)

Freezing process used to produce a frozen strawberry product leads to some changes in nutritional content. The most significant change that occurs is when sugar is added to the frozen product. As shown in Figure 2.18 below, the addition of sugar

changes the caloric content of the strawberries as well as the amount of sugar and total carbohydrate found in the product. Frozen processing does not have an impact on sodium, fiber, Vitamin A, or Calcium (Harmit, 2005).

Nutrients (221g)	Fresh	Frozen Unsweetened	Frozen Sweetened
Calories	71	77	172
Sodium	3 mg	4 mg	3 mg
Total Carbohydrate	17 g	20 g	47 g
Dietary Fiber	4 g	5 g	4 g
Sugars	10 g	10 g	42 g
Vitamin A	0 %	2 %	1 %
Vitamin C	217 %	152 %	146 %
Calcium	3 %	4 %	3 %
Iron	6 %	9 %	6 %

Figure 2.18: Nutritional information for fresh, frozen unsweetened and frozen sweetened strawberries

Source: Harmit (2005)

It is interesting to note the impact of frozen processing has on the Vitamin C content of the strawberry. Research suggests that there are changes in quality parameters such as anthocyanin content, total ascorbic acid, and total soluble sugars during cool storage (Cordenunsi, 2005). However, in this study the changes were more closely linked to the differences in cultivar than the process of cool storage. Another study found that the loss of ascorbic acid occurs during the first 15 days of storage (Sahari et al., 2004). Furthermore, the best storage temperatures for strawberries were found at either -18°C or -24°C to preserve the qualitative characteristics of the berry (Sahari et al., 2004).

In the study conducted by Ayala-Zavala et al., (2004), higher antioxidant capacity, total phenolics, and anthocyanins were found in strawberries stored at 10° or 5°C in comparison to 0°C. This finding suggests that the antioxidant capability of strawberries is reduced after the strawberry undergoes frozen processing. Another conclusion from the study was that shelf-life was longer at 0°C than either 5° or 10°C based on overall quality of the fruit. Since most frozen strawberries are processed at

0°C, it appears the industry has chosen to forgo antioxidant capacity in exchange for longer shelf life.

Nutritional information for freeze-dried strawberries is a little convoluted. Shown in Figure 2.19 are the two nutritional facts currently available for freeze-dried strawberries. The first column shows the nutritional facts for a freeze dried strawberry product intended to be reconstituted before consumption. The second column is the nutrition information for the novelty item called "Astronaut Strawberries" intended to be consumed before reconstitution (Harmit, 2005).

	Freeze Dried, ½ cup (9.5g)	Freeze Dried 0.5 oz
Calories	36	50
Dietary Fiber	1.4 g	n/a
Sugar	4.7 g	n/a
Vitamin C	47%	100%

Figure 2.19: Nutritional information for freeze-dried strawberries

Source: Harmit (2005)

When comparing the nutritional information for one cup of fresh strawberries we can see that the reconstituted freeze-dried strawberries maintain approximately the same amount of calories, fiber and sugar. However, there is a drastic reduction in the amount of vitamin C in the reconstituted product (Harmit, 2005).

In a study done by Pirker et al., (2002) it was found that there was an inverse relationship between the free radical content of freeze-dried fruit and the water content of the fresh fruit; the higher the water content in fresh fruit the lower the content of free radicals in the freeze dried fruit. Furthermore, Pirker et al., (2002) concluded that the free radical levels in freeze-dried fruits were approximately ten times higher than in frozen samples. This suggests that there must be free radical generation in the freeze-drying process. The generation of free radicals could create impact on the amount of Vitamin C available in a freeze-dried product (Harmit, 2005).

Strawberries have proven to be a rich source of Vitamin C for our health benefits. Furthermore, there have been a lot of researches of the health benefits in strawberries. Unfortunately there seem not to be any research on the consumption of strawberries and local jackfruits for health. In the current literature there seems to be limited information about nutritional changes that occur during the processing of those fruit. Although strawberries are available all year round and jackfruits occur seasonally, a majority of individuals consume processed strawberries and jackfruits over fresh strawberries and jackfruits for many months in a year. Current research suggests that freezing temperatures and length of time frozen have different impacts on the antioxidant levels of those fruit. More research should be conducted to examine the best combination of the two in order to maintain the nutritional integrity of those fruits. In addition, researchers should look at the content changes inside of strawberries and jackfruits microstructure changes during processing.

Other than that, there seems to be very little information available about the freeze-drying processing technique which causes a reduction in Vitamin C in those fruits. Furthermore, it is necessary to find out those fruits microstructure that affect their nutritional changes after undergoing this process. Lastly, to increase the demand for a freeze-dried product, the industry should focus on making it economically feasible to replace fruit flavoured pieces with real strawberry pieces in snack bars.

As a result of this research, there are many factors that consumers should be aware of when consuming strawberries and jackfruits. First of all, consumers should be aware of the health benefits that can be received by including strawberries and jackfruits as a part of their diet. The best source for these health benefits come from fresh strawberries and jackfruits. However, when fresh ones are not available it is appropriate to supplement the diet with frozen or freeze-dried strawberries and jackfruits. Secondly, consumers should be aware that nutrition changes due to processing. Both frozen strawberries or jackfruits and freeze-dried strawberries or jackfruits lose vitamin C, with the latter losing the most.

Currently, it is unknown what the impact of these processes is on the antioxidant capacity. However, it appears that both processes lead to a reduction in the antioxidant capacity due to the micro structural changes of those fruits during freezing. At this point there is not enough information to conclude which process leads to a greater destruction of antioxidants. A third and final investigation is to make the consumers aware when purchasing frozen berries or jackfruits. Diabetics and others who must monitor their sugar or carbohydrate intake should be aware of the added sugar in frozen strawberries or jackfruits and replaced them with new freeze-dried products in our local market soon.

2.9 X-RAY MICRO-COMPUTED TOMOGRAPHY TECHNIQUES

The use of freezing as a preservation technique is well established for many commodities as well as processed foods. Freezing often results in substantial textural damage caused by the growth of ice crystals within the delicate structure either present naturally or created during processing. However, freezing can also generate a textured product from an amorphous protein paste or slurry (Mousavi et al., 2005 and Lawrence et al., 1986). In myco-protein, which composed of Fusarium venenatum mycelium set with egg albumin, freezing is considered necessary to produce the required fibrous texture. The process is possibly analogous to the “freeze texturization” used for some food protein materials, such as kori-tofu (Mousavi et al., 2005 and Lawrence et al., 1986). Freeze texturization relies on the compression of the material between ice crystals, which result in the polymerization of proteins into fibers.

Understanding the relationship between the freezing conditions and the size of ice crystals formed is critical in controlling product quality and texture. Observation of the ice crystal size may be direct or indirect. Direct observation methods include cryo-scanning electron microscopy (Mousavi et al., 2005 and Russell et al., 1999), cold microscopy (Mousavi et al., 2005 and Donhowe et al., 1991), and confocal laser scanning microscopy (Mousavi et al., 2005 and Evans et al., 1996). Indirect methods such as freeze substitution (Mousavi et al., 2005; Bevilacqua et al., 1979, and Martino and Zarizky 1998), freeze fixation (Mousavi et al., 2005 and Miyawaki et al., 1992), and freeze drying techniques (Mousavi et al., 2005; Fayadi et al., 2001 and Woinet et al., 1998a, b) followed by sectioning have been used. Indirect methods assume that the

original morphology is maintained during the sectioning into thin enough layers to allow microscopic methods to be used.

These techniques, thus, have the disadvantages that the microstructure might change during cutting. They are also limited to observation of a thin layer of material. In addition, conventional optical or electron microscopy allows visualization of only two-dimensional (2 -D) images or thin slices (Mousavi et al., 2005). In most cases, conclusions about the original three-dimensional (3-D) ice structures cannot be made on the basis of 2-D information unless a suitable stereological method is applied to relate the 3-D structure to the measured 2-D structure (Mousavi et al., 2005; Underwood 1970, and Xu and Pitot 2003). Three-dimensional information on ice crystal structures can be achieved either by first obtaining the 2-D information and then using image reconstruction techniques or directly from 2-D information by appropriate software.

Ueno et al., (2004) and Do et al., (2004) applied a Micro-Slicer Image Processing System (MSIPS) to observe 3-D ice crystal structure in frozen dilute solution and beef. They used algorithms to reconstruct the 3-D image based on 2-D cross-sections which resulted from multiple-slicing of a frozen sample with the minimum thickness of 1 μm. The method is cumbersome and possibly unreliable, as the ice structure itself can be altered by the preparation technique and the distance between the slices may be too coarse to avoid loss of 3-D information.

The X-ray micro-computed tomography system (XMT) allows visualization and measurement of complete three-dimensional object structures without sample preparation or chemical fixation. It uses a combination of X-ray microscopy and tomographical algorithms, based on the contrast in X-ray images generated by differences in X-ray attenuation (absorption and scattering) arising from differences in density of material within the specimen. X-ray passes through specimens that is rotated in many different directions and yields an image that displays differences in density at thousands of points in the 2-D slices through the specimen. Many contiguous slices, each of a certain finite thickness of around 18 μm, are generated this way and stacked up to reconstruct a 3-D distribution of material density within the object (Mousavi et al., 2005). The technique has so far been successfully applied to a wide range of materials

such as rock, bone, ceramic, metal, and granules (Farber et al., 2003 and Salvo et al., 2003). The aim of this study is to demonstrate the potential of XMT (Figure 2.20) as a non-destructive technique for the study of the internal microstructure of freeze-dried strawberry and jackfruit (Nurzahida et al., 2010).

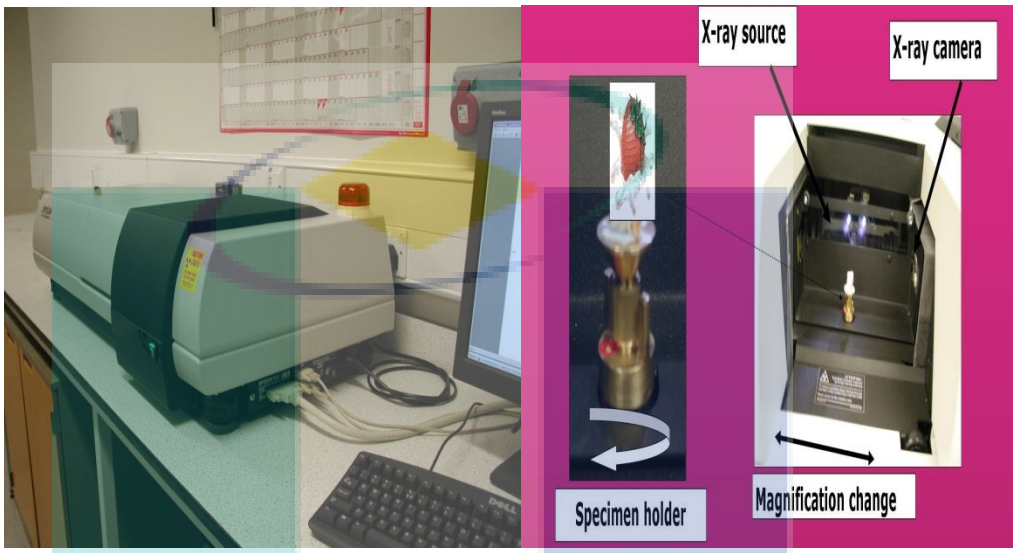

Figure 2.20: Overview of XMT system

2.10 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Chromatography is a separation technique of two or more components in a mixture due to the differentiation in interaction (chemically or physically) or adsorption between mobile phase and stationary phase. In the early 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressurized liquid chromatography began to be used to increase flow through time, thus reducing purification times of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have a constant flow rate or a constant pressure was debated (Marsin et al., 2008 and Braithwaite et al., 1999).

High Performance Liquid Chromatography (HPLC) was developed in the mid 1970's and quickly improved with the development of column packing materials and the additional convenience of on-line detectors. In the late 1970's, new methods including reversed phase liquid chromatography allowed for improved separation between very similar compounds. By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far beyond the previous techniques (Marsin et al., 2008 and Lunn et al., 2000). Computers and automation were added to the convenience of HPLC. An improvement in types of columns and thus reproducibility was made.

In many analytical laboratories, HPLC has become an indispensable technique for the analysis of samples, the determination of physical constants and the isolation of purified components from complex mixtures. Now, HPLC has found broad acceptance as the analytical technique of choice in many scientific and application-oriented areas such as life sciences, food, synthetic polymers and environmental chemistry. In addition, in order to meet legal requirements in application areas such as pharmaceutical and clinical chemistry, HPLC analysis protocols are standardized and validated (Marsin et al., 2008 and Synder et al., 1997).

2.11 UV SPECTROPHOTOMETER

A spectrophotometer is an instrument used to measure the intensity of light passing through a sample due to absorption or excitation. Spectrophotometers are utilized by researchers to determine the absorbance of a sample, ultimately allowing for the determination of color, concentration, or other pertinent information (Danny et al., 2003). A wide array of spectrophotometers exists, providing varying wavelengths, measurements, and source lamps. The use of spectrophotometers covers just about any industry, from beverage manufacturers to biochemists.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

The research work focuses on the achievement of the conceptual study, equipment development, laboratory experimental work, analyze and completion of the project. The detailed procedure of the experimental work is discussed throughout this chapter. The purpose of the experimental work phase is to understand the required for the experiment. This phase will include the two major steps which are requirement laboratory testing, and data analysis. Besides that, the analysis is being done to get the data for the research. It is to be noted that this study was carried out under conditions of lab scale processing and the main experimental studies were done to the strawberry samples while for jackfruits samples the materials and methods only applicable to image visualisation and nutrient analysis: Freeze-drying of a thick layer (about 15mm high) of both fruit products previously cut and frozen in stainless steel trays (loading density about 250g/m^3). The main activities involved in this research were summarized and illustrated in Figure 3.1 and Figure 3.2.

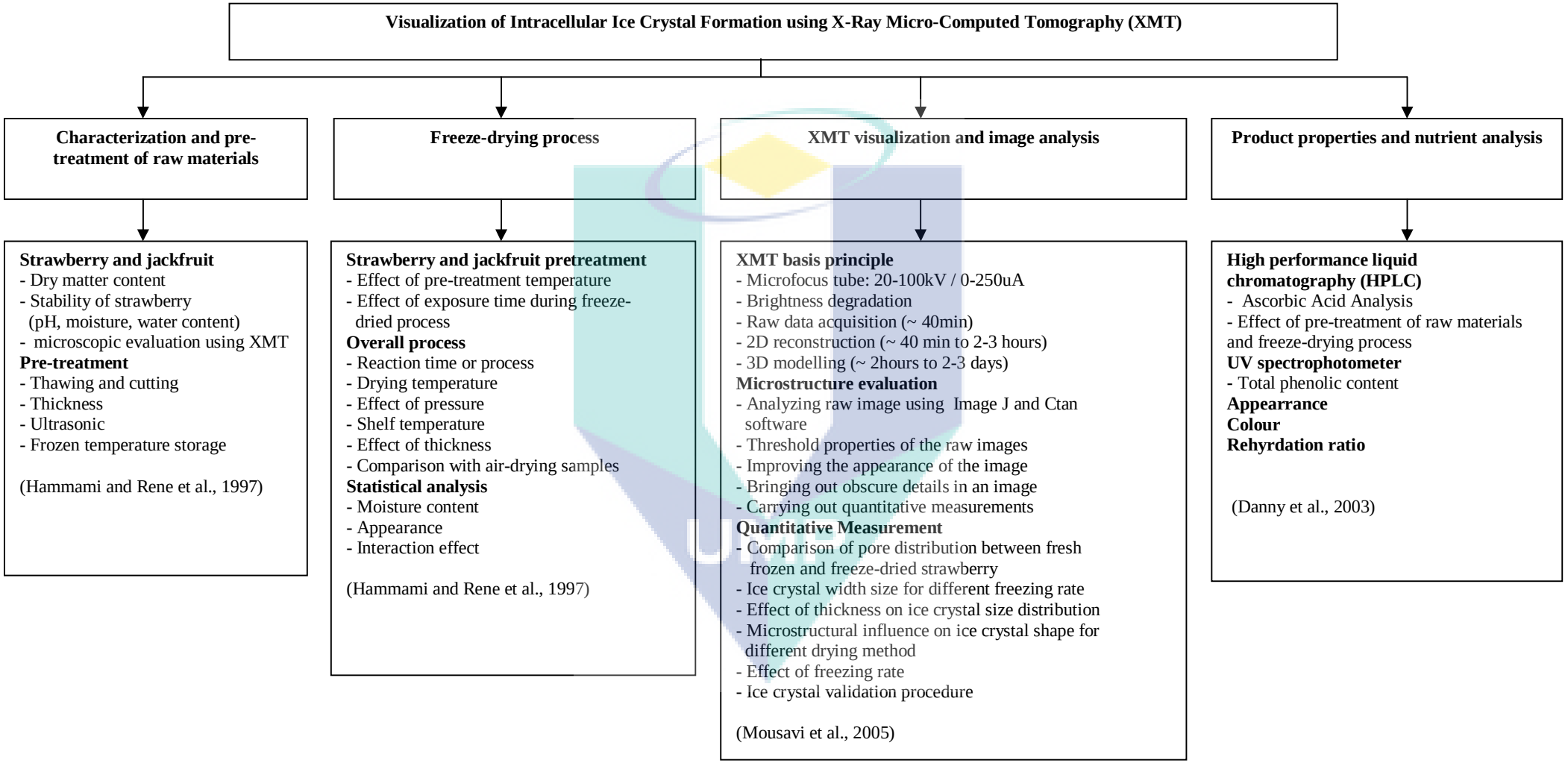

Figure 3.1: Summarization of main research activities

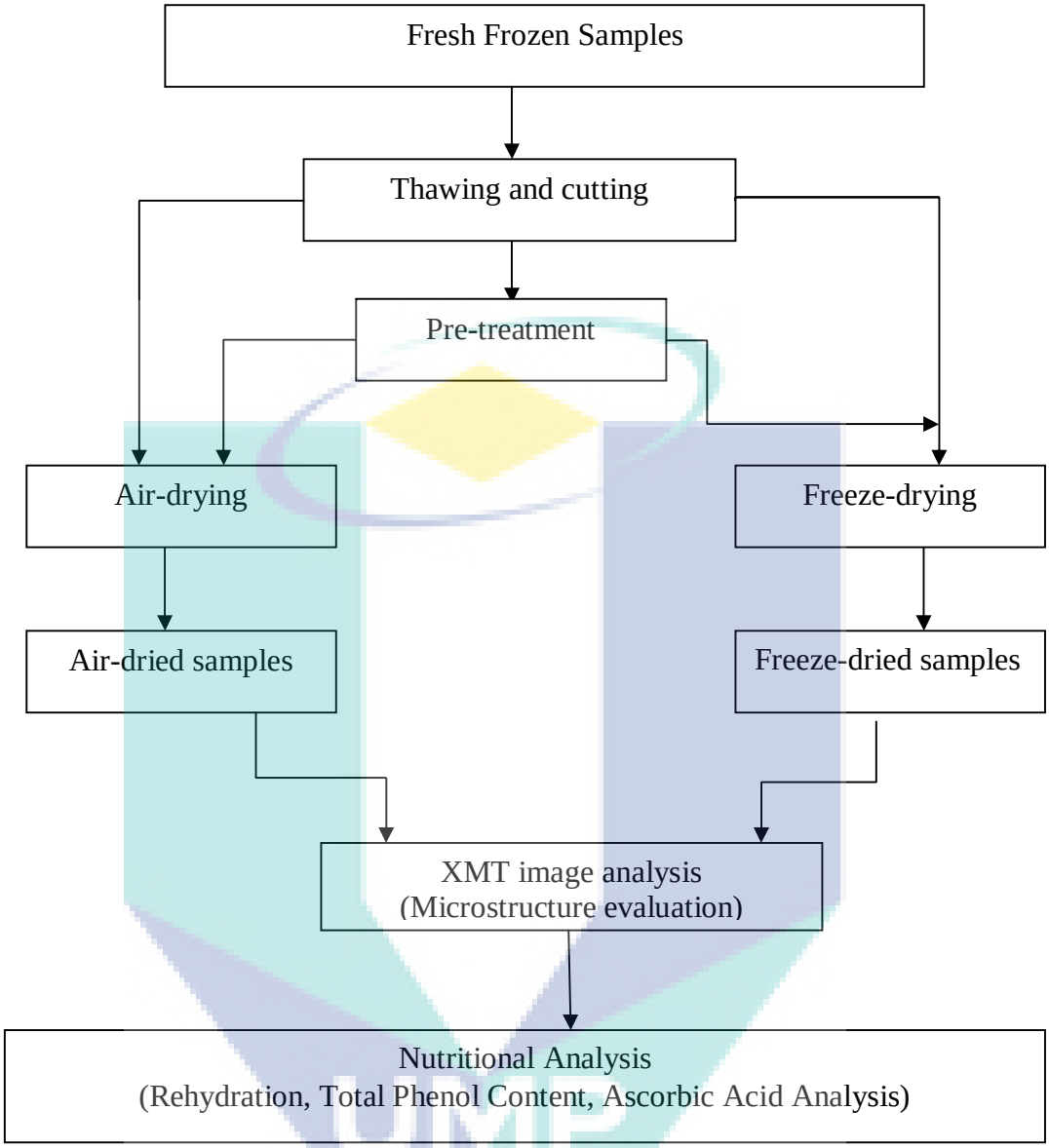

Figure 3.2: Flow chart diagram of experimental set-up

3.2 SAMPLES PREPARATION

Strawberry fruits were collected from an orchard in Cameron Highlands, Pahang, Malaysia in October 2008. Fresh strawberries were transported to the Analytical Lab of Chemical Engineering Department of University Malaysia Pahang on the same day of collection and were immediately cut into various thicknesses and jackfruits of the brand “Carrefour Stores” were bought in sufficient quantity and had

been cut using the appropriate apparatus. Before freeze drying and characterization of ice crystal formation, strawberry and jackfruit had been cut into different thickness (5mm, 10mm and 15mm). It had been ensured that during the cutting of the outer structure of the strawberries and jackfruits were not destroyed as it had been judged not acceptable for the damaged samples to be used in the characterization of structure by the micro CT apparatus and these would lead to a report of an erroneous data. The illustrated way of cutting the strawberry and jackfruit samples shown in Figure 3.3 and 3.4.

Figure 3.3: Slice of strawberry samples in different thickness

Figure 3.4: Slice of jackfruit samples in different thickness

Then all the strawberry and jackfruit pieces had been frozen in different freezing rate which is in normal refrigerator condition (4^{o}C) , slowly freezing at -20^{o}C and rapidly freezing at -80^{o}C (Hammami et al., 1997).

3.3 FREEZE DRYING PROCESS

Biotron Clean Vac 12 Freeze Dryer had been used in order to freeze dried the strawberry samples. This type of freeze dryer is a mechanically refrigerated freeze dryer in which throughout the process of sublimation it can remove up to 99% of the moisture of a frozen product. The samples were kept frozen under vacuum condition throughout the process. This freeze drying process is very effective in preserving the crystalline structure and it prevents future degradation of the product. The freeze drying process is needed by the products that used preservation techniques in which they are biologically or chemically active at room temperature, required storage at temperature above freezing and subjected to physical degradation.

Figure 3.5 below shows the Biotron Clean Vac 12 Freeze Dryer is a lab scale freeze dryer using the lyophilization phenomena. The cylindrical shape transparent acryl chamber is used for dry chamber and stainless steel petri dishes are used with rack system to dry the frozen specimen. The Clean vac system has a very large size cold trap (temperature -85^{o}C) that it can be used for pre freezer and it does not need the exclusive pre freezer system. The microprocessor governs the whole system process which monitors the temperature with pt100 temperature sensor and vacuum level with pirani gauge. The microprocessors automatically decides the leak condition from the vacuum level and gives warning to the LCD display in order to prevent the damage of vacuum pump by overflow of the molten specimen. Furthermore, the microprocessor periodically stops the vacuum pump and measures the change of vacuum level to find out the end of freeze-drying procedures.

Lyophilization is the most effective method to preserve living cells, whole blood, vaccines, biological specimen, vegetables, fruits, meat, fish and other general foods. The powerful compressor (1Hp) with CFC-free refrigeration system can pull

down the temperature of the cold trap to -85^{o}C. The removable front of the panel provides easy access to refrigeration system and pump maintenance. High capacity refrigeration system (maximum 12 Liter) in this equipment ensures rapid cooling. The vacuum pressure is measured by Pirani vacuum gauge. The microprocessor governs the whole system and all the parameters are displayed in the LCD display. Self diagnosis algorithm for checking the end of the freeze-drying procedure in which stopping the vacuum pump at each hour and check the pressure changing during a minute. If the value of pressure change is limited in the pre determined range, the controller automatically displays the end of the drying procedure on the LCD panel. The heater plate can be installed on the rack for petri dishes in order to accelerate the drying speed. LCD display of the equipment shows the vacuum pressure, chamber (trap chamber: 310D X 300H/mm) and trap temperature and also other processing parameters.

Figure 3.5: Biotron Clean Vac 12 freeze dryer

The first step in freeze drying was to freeze the samples (strawberries and jackfruits) to a minimum of -80^{o}C. After the samples were frozen then only the moisture could be extracted through sublimation. Sublimation is the process where the ice (solid) is converted into vapor (gas), by passing the liquid stage. The moisture inside the sample is converted into gas without damaging the crystalline structure and this protects the integrity of the samples (Hammami et al., 1997). Second step of the freeze drying process was the migration of moisture from the samples. The BioTron Freeze Dryer showed in the Figure 3.5 done two things to facilitate the migration. This freeze dryer created a pressure free zone using a vacuum in order to extract the non-condensable vapors in the chamber. The moisture then flowed out easily from the samples. Moisture had been pulled away from the samples by cooling down the condenser to -45^{o}C. Then the shelf temperature of the drying cycle was set to be at 50^{o}C and these samples had been dried for 24 hours under vacuum condition at 0.1 Torr. All in all, the entire freeze -drying process which includes four major steps: freezing, vacuuming, heating and condensing were illustrated in Figure 3.6.

After the freeze drying process had finished, the weight of the dried sample then was measured (Hammami et al., 1997). The moisture contents of the strawberries and jackfruits samples were then obtained using the following equation (3.1):

$$\text{Moisture content} = [M_w - M_d]/ M_w \quad (3.1)$$

Where; M_w Mass of wet sample (before freeze drying)

M_d Mass of dry sample (after freeze drying)

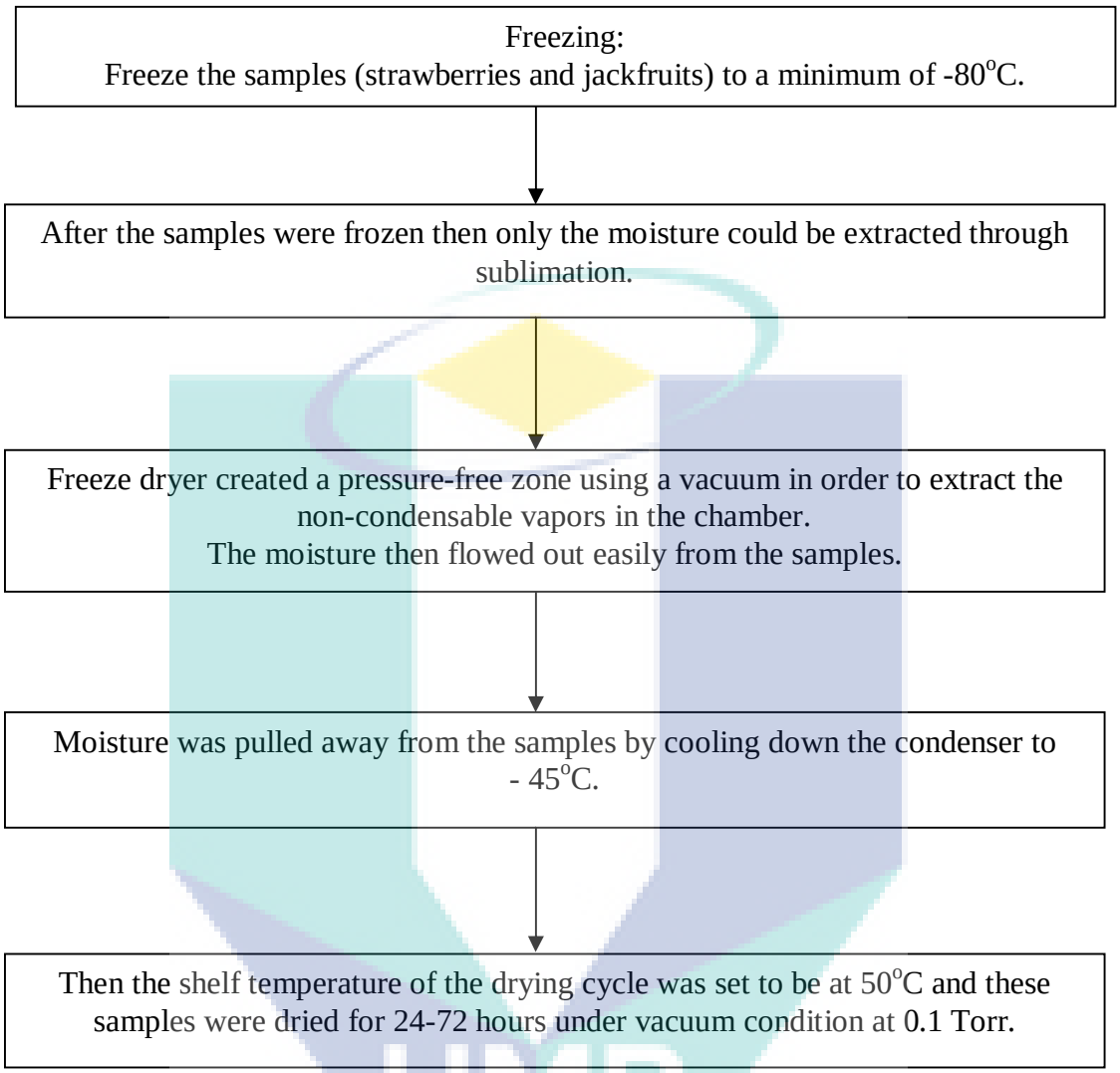

Figure 3.6: Process flow of freeze-dried

3.4 X-RAY MICROTOMOGRAPHY PROCESS

The white painted metal sheet of Skyscan 1072 X-ray microtomograph was used in the present study and it is shown in Figure 3.7. It also includes a chamber where the sample was being placed throughout the analysis. The lead shield outside the chamber was kept closed in order to prevent any X-rays to escape. It is powered by electricity with appropriate software attached to a computer drive. It has to be connected to the computer during operation because an exchange of data between both is needed during

the analysis period. It only responds to the command when it is being connected to each other. A flashing red light at the back of this apparatus will be lit automatically when it detects that X-rays are generated from this equipment. The sample was placed on the sample holder and it had been fixed securely into the right position before the operation could take place. The X-rays is produced by the apparatus and directly spot onto the sample. The sample is perpendicularly rotated about its long axis and the images are directly shown as grey scale image on the computer. The photographs of the sample at different positions are taken and saved to the computer drive. The images then being reconstructed using software (Nrecon) and a plane image obtained is further used in the analysis of the sample that is performed by the analysis software (CTan analysis) (Mousavi et al., 2005).

Figure 3.7: Skyscan 1072 x-ray microtomograph

3.4.1 Characterization of Ice Crystal Formation

The characterization of ice crystal formation in strawberry and jackfruit had been done using the Skyscan X-ray microtomograph. For strawberries and jackfruits samples, the dried sample had been used to characterize the structure of ice crystal formation. Microtomograph which is attached to the computer that had the appropriate software for this equipment was switched on. After the microtomograph had been switched on then only the software could be run by the computer. Then the samples had been put in the microtomograph sample's place when the sample chamber's door was opened and it had to be closed automatically before the x-ray was started by the equipment. All opened and closed functions of the door were being controlled by the software that was attached to that equipment. The ageing process started for the one-time of the equipment used in a day and it took approximately 15 minutes to complete. The samples then were being scanned and the voltage required for the scan was 50 KV with the current 96 μA. No filter condition was being chosen so that the voltage applied was within the range that appropriate for those samples. The samples area to be scanned was chosen by the software that had the tools to resize the samples. It would give a maximum view of the samples and the resizing order was 14X and it was applied to all samples. The software could lift up and down the length of the samples which would fill the view that was being scanned. The image for each 0.9^{o} angles of the samples from microtomograph that had been set was taken and the process took approximately 30 minutes via the software (Mousavi et al., 2005).

After the process had finished, the raw images collected had been reconstructed using the "Cone and Beam" reconstruction software that would give a set of circular two-dimensional plane image. Then the reconstructed images would be applied to the analysis software called "CTAn" and "Image J". The quantification of the ice crystal formation (void) and density threshold for the samples would be obtained throughout this analysis. A large amount of other data was obtained but had deemed to be irrelevant to the present study and had been overlooked throughout this study.

3.5 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS

3.5.1 Ascorbic Acid Analysis

Samples were homogenized for 1 min at maximum speed in a blender. The homogenate (1-5 g) was added to 20ml of 4.5% metaphosporic acid and vortexed. Extracts were centrifuged for 15 minutes at 20^{o}C. The supernatant was filtered through a Whatman no.1 filter and diluted to 25 ml with the 4.5% metaphosporic acid. Ascorbic Acid (AA) concentration was measured according to established methods with minor modifications (Danny et al., 2003). Analysis was performed using Agilent HPLC. Reverse-phase separation was attained using an appropriate column. The mobile phase was nanopure water brought to pH 2.2 with sulfuric acid. The flow rate was 0.5mL/min, the detection wavelength was 245nm. Samples aliquot were filtered through a 0.45micrometre poly (tetrafluoroethylene) filter prior to injection. All samples were run in triplicates.

3.6 UV SPECTROPHOTOMETER ANALYSIS

3.6.1 Total Phenolics Analysis

3.6.1.1 Extraction of Phenolics

The extraction of phenolics contents from strawberries and jackfruits had been done by Danny et al., (2003) with minor modifications. Samples were homogenized for 1 min at maximum speed in a blender. A 3g aliquot of the homogenized sample was then transferred to polypropylene tubes and extracted with 40mL of a mixture containing acetone, water, and acetic acid (70:29.5:0.5 v/v). Samples were vortexed and allowed to stand for 1h at room temperature to allow for complete solvent extraction. Extracts were centrifuged for 15 minutes at 20^{o}C. The supernatant plus a subsequent 15mL water rinse was filtered through a Whatman no. 1 filter, after which the filtrate was concentrated using a rotary evaporator under partial vacuum at 117^{o}C. Samples were concentrated to 25mL and were brought up to a total volume of 30mL with

Nanopure water. Extractions were repeated on three independent samples of the initial homogenate to give triplicate readings (Danny et al., 2003).

3.6.1.2 Measurement of Total Phenolics (TP)

Total phenolics concentration was measured using the establish methods by Danny et al., (2003) with minor modifications. The Folin-Ciocalteu assay method had been used in this study. Briefly, 5mL of Nanopure water, 0.5-1.0mL of sample, and 1.0mL of Folin-Ciocalteu reagent were added to a 25mL volumetric flask. The contents were mixed and allowed to stand for 5-8min at room temperature. Next, 10mL of 7% sodium carbonate solution was added, followed by the addition of Nanopure water filled to volume. Solution were mixed and allowed to stand at room temperature for 2 h. Samples aliquots were filtered through a Whatman 0.45 micrometre poly (tetrafluoroethylene) filter prior to the determination of total phenols concentration using a UV spectrophotometer monitoring 750nm. TP content was standardized against Gallic acid and expressed as milligrams per liter of Gallic acid equivalents (GAE). The linearity range for this assay was determined as 0.5-5.0mg/L GAE (R2 =0.9980), giving an absorbance range of 0.050-0.555 AU (Danny et al., 2003).

3.7 AIR-DRYING

A tray was used to put strawberries and jackfruits pieces in the centre of a fan oven pre-heated to 60^{o}C. The samples were briefly removed at regular intervals and their mass determined over time until, 10% w/w moisture content was reached. The moisture content at each time point was calculated from the total initial moisture content and the mass loss during drying. It was assumed that all mass loss was due to water removal. All drying experiments were carried out in triplicates (Brown et al., 2008).

3.8 APPEARANCE

The appearance of the freeze-dried products was assessed by visual comparison to a reference of fresh strawberry and jackfruit samples (Rene et al., 1993). Special attention was paid to shrinkage. The scoring system was none (product similar to fresh fruits), poor, and considerable (shrivelled).

3.9 REHYDRATION RATIO

The capacity of freeze-dried strawberries to rehydrate was measured by weighing about 1 g of freeze-dried product after soaking in distilled water (50^{o}C) at different times. The rehydration coefficient (CR %) was defined as the ratio of the amount of water taken up over the total amount of water removed by freeze-drying. The maximum rehydration coefficient is obtained when soaking the product in warm as water does not change its weight significantly (Hammami et al., 1997).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 CHARACTERISATION OF FRESH STRAWBERRIES

The characterisations of fresh strawberries are presented in Table 4.1. The values of each characterisation listed, remain within the ranges found in the literature. The 10.8% dry matter content for this study samples is consistent with the values reported by Skrede (1980) for twelve strawberry varieties (9.7 to 11.3%). The soluble solid content of 8.5% measured in this investigation is also within the range of 8.l to 10.9% found by the same author (Hammami et al., 1997, and Skrede, 1980). The pH of 3.52 is included in the range of 3.34 to 3.57, determined by Skrede (1980). The dry weight of the strawberries in these experiments averaged about 10.8 % of the initial fresh weight and changed only slightly during storage. The high levels of water loss in the strawberries from the 3 hours delay to cooling treatment tended to mask real losses on a fresh weight basis of some constituents; in some cases seeming to show no difference, or even greater retention of some constituents compared to the strawberries cooled without delay after transferring to the analytical lab. Although it might be argued that the compositional values expressed on a fresh weight basis represent the actual concentrations that would be experienced by consumers, the results have expressed the compositional data on a dry weight basis as well in order to illustrate the actual losses that occurred in certain constituents irrespective of the concentrating effect imposed by water loss. The point may again be made that, since the cooling and storage conditions were not identical for all treatments, greater losses of individual constituents must have occurred primarily during the transfered of raw material to the analytical lab of Universiti Malaysia Pahang.

The water loss had a negative effect on the strawberry fruit appearance, leading to shrivelling and a dull appearance of the strawberry epidermis. The maximum permissible water loss for strawberries before marketability is impaired has been reported to be approximately 6% (Robinson et al., 1975). Strawberry pH was little affected by the delay treatment, despite some indication that there were greater losses of titratable acidity (expressed as citric acid equivalents) in the strawberries with 3 hours delay before cooling. Acidity levels have been reported to decrease in overripe strawberries (Woodward, 1972). Soluble solids content was also higher in those fresh strawberries samples that were delay to undergo cooling treatment than the other samples that were not delay to cooling which had lower soluble solids content on a dry weight basis.

The physical and chemical quality characteristics of strawberries that were measured in this study showed substantial variability among fresh strawberries that were immediately treated with cooling condition and the sample that were delay to undergo cooling process. The result of the simulated handling scenarios were imposed on those samples. Despite these differences, when cooling was delayed for 3 h at 4°C, the strawberries were significantly softer, more shrivelled, had less attractive color, and the acidity, soluble solids contents levels were lower than in fruit that were more quickly cooled. These differences were all apparent after seven days storage at 4°C, conditions simulating normal transportation and marketing times with optimum temperatures for strawberries. Thus, a rather modest and probably not unusual in commercial handling, the delay time between harvest and cooling of strawberries was shown to have significant negative effects on appearance, nutritive value, and presumably acceptability, that would be expected to persist at the consumer level. This study seems to affirm the assumption made by Kader (1990) that rapid cooling, in addition to proper storage and transport temperatures, is critical for maintaining strawberry quality. The results obtained in the texture study are difficult to compare to those found in the literature. The principal reasons for this are the different systems used to express the results and the wide variety of measurement methods employed. In addition, product texture strongly depends on its degree of ripeness (Szczesniak and

Smith, 1969) the variety of the strawberry and the measurement method (Nunes et al., 1995; Planton, 1993 and Skrede, 1982).

Table 4.1: Comparison of the main properties of the fresh strawberries used in this work with the values found in the literature for strawberry. The standard deviation measurements are indicated in parantheses

Comparison	This study	Literature	Reference
Dry matter content(%)	10.8 (0.96)	9.8	Hammami et al.(1997)
		9.6 to 12.3	Hammami et al.(1997) and Simatos et al.(1974)
		9.7 to 11.3	Hammami et al.(1997) and Skrede (1980)
		9.2 to 11.6	Hammami et al.(1997) and Skrede (1982)
pH	3.52 (0.06)	3.49	Hammami et al.(1997)
		3.34 to 3.57	Hammami et al.(1997) and Skrede (1980)
		3.45 to 3.67	Hammami et al.(1997) and Skrede (1982)
Soluble solids(%)	8.5 (0.8)	7.9	Hammami et al.(1997)
		8.1 to 10.9	Hammami et al.(1997) and Skrede (1980)
		9.2 to 11.6	Hammami et al.(1997) and Skrede (1982)
Water Activity A_w	0.98 (0.003)	0.99	Hammami et al.(1997)
Energy of rupture (10^{-3} J)	6.25 (0.99)	6.27	Hammami et al.(1997)
Glass transition temperature T_g (^oC)	-33.4 (1.7)	-34.3	Hammami et al.(1997)
		-34.1	Hammami et al.(1997) and Maltini et al.(1976)
		-33.5 to -39.1	Hammami et al.(1997) and Levine et al.(1989)

4.2 PORE DISTRIBUTION IN FREEZE-DRIED STRAWBERRY

Analysis of the ice crystal size in strawberry samples had been done using the microtomograph (CTan) software which can represent the internal microstructure and void distribution within the samples. The procedure had been adapted to both analyzing works in order to obtain the representative result. For strawberry analysis, the samples had been looked at three different points which were the top, middle and bottom of each sample. This CTan software had been run on these samples to obtain statistical data for the whole samples. This software gave the difference for various types of samples when the threshold had been done to the sample and it did not appear at the same time.

The details study on microstructure changes had been done in strawberry samples only while for jackfruit samples; the analysis is being limited to image validation and nutrient analysis only. Further investigation exhibit the x-ray shadow images of strawberry samples and a typical reconstructed 2D horizontal slices had been implemented in order to show clearly the open cell structure of the ice crystal formation. In a given single scan, the ratio of the height of the sample to the resolution of the scan represents the total number of 2D horizontal images that can be reconstructed, without any physical slicing required. In this case, the scan generated a total of 1500 2D horizontal images.

Size measurements were made directly from photomicrographic prints. A rectangular pattern was laid over the print to provide a statistically analysis. The projected area of an ice crystal was determined by tracing the perimeter of the ice crystal on a digitizing Image J and CTAN software connected to a micro computer. The size of each ice crystal, defined as the diameter of a circle of area equivalent to the projected area of the ice crystal, was then calculated by that software. Ice crystal size distributions were generated by grouping ice crystal sizes into 10 to 50 classes; the classes were either linearly or logarithmically distributed, and the number of classes depended on the total number of ice crystal sized.

Distributions were fit to linear or normal type of distributions. The number weighted arithmetic mean diameters and the standard deviation of the log normal distribution were also calculated. Normally only high magnification photographs were digitized, but when samples exhibited a relatively wide ice crystal size distribution, larger ice crystals were digitized using the low magnification photographs. This technique resulted in more of the larger ice crystals being sized that would be possible using only high magnification photographs, thus improving the accuracy of the distribution at larger ice crystal sizes and reducing the number of crystals required to obtain an accurate ice crystal size distribution. Analysis of at least 50 ice crystals from a sample ensured an accurate representation of ice crystal size distribution was not changed significantly. This number was determined as a result of performing cumulative image analyses on increasing numbers of ice crystals within a sample. Recommended numbers of void measurement for size distribution analyses are similar; at least 50 measurement are recommended for each modal class and at least 7 mesurement for each class. 2D quantitative measurement and information including void cell count, number-mean cell size, total cell area and relative cell area were extracted from the reconstructed 2D horizontal slice images directly from the T-View image and Nrecon software that were coupled with the X-ray scanner.

4.3 COMPARISON BETWEEN FRESH FROZEN AND FREEZE-DRIED STRAWBERRY

Figure 4.1 shows the shadow of a fresh strawberry ($4^{o}C$) sample imaged by Skyscan x-ray micro tomography. The microstructures of this fresh strawberry were investigated by x-ray. The image after this sample had been reconstructed using Nrecon software to view the microstructure is being visualized in Figure 4.2. The summary of the statistical analysis for this sample is illustrated in Table 4.2.

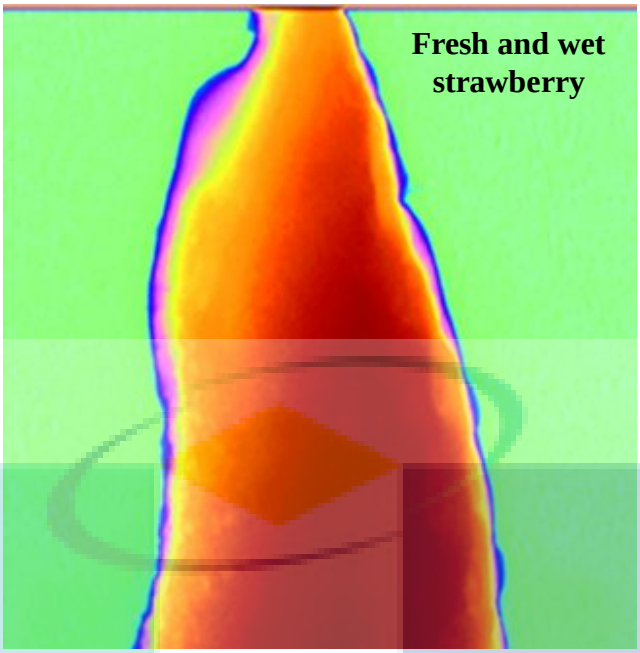

Figure 4.1: Raw sample of fresh strawberry freezing under 4^{o}C as imaged by x-ray micro-computed tomography

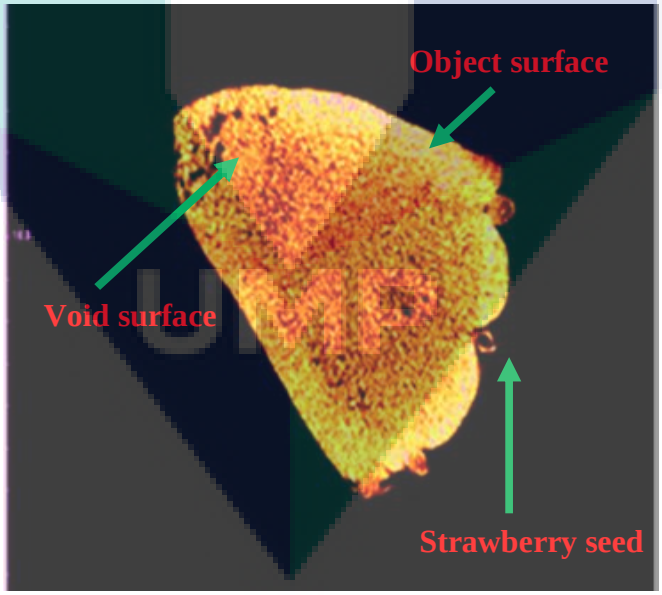

Figure 4.2: Typical reconstructed cross-section of the fresh strawberry

Table 4.2: Summary of statistical data for fresh strawberry

Strawberry (Object)	
Pixel size (um)	37.74
Void volume,TV (mm^3)	422.05
Object volume,BV (mm^3)	418.02
Percent object volume,BV/TV (%)	99%
Void surface,TS (mm^2)	645.92
Object surface,BS (mm^2)	760.44
Object surface / volume ratio,BS/BV (1/mm)	1.82

Microstructures of fresh and frozen strawberries were investigated by x-ray. The fresh strawberry shown in Figure 4.2 produced shadow images for the samples and no differences or void were distinguishable. Although there was some small intercellular voids existed but it was just 1% of the total 99% volume in the strawberry. While for the freeze-dried strawberry (-80^{o}C) sample it was illustrated in Figures 4.3 and 4.4. The statistical data for these freeze-dried samples is summarized in Table 4.3. Thus, both images shows the differences between fresh (4^{o}C) and frozen strawberry (-80^{o}C). Different microstructure could be observed by both fresh and frozen samples. The x-ray image clearly showed that the voids left by the ice crystals and in addition, differences in microstructure of the ice crystals were seen in different axial of cross-sectional area of the samples, showing that the ice crystals distribution was influenced by the rate of the freezing condition.

Table 4.3: Statistical data analysis summarization for frozen strawberry using freeze dried technique

Strawberry (Object)	
Pixel size (um)	18.23
Void volume,TV (mm^3)	15.87
Object volume,BV (mm^3)	11.41
Percent object volume,BV/TV (%)	72%
Void surface,TS (mm^2)	770.49
Object surface,BS (mm^2)	878.39
Object surface / volume ratio,BS/BV (1/mm)	76.99

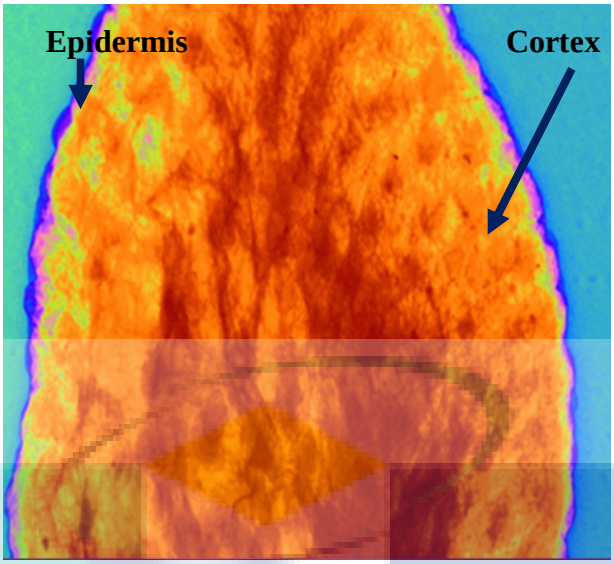

Figure 4.3: Dried strawberry after freeze-drying imaged by X-ray

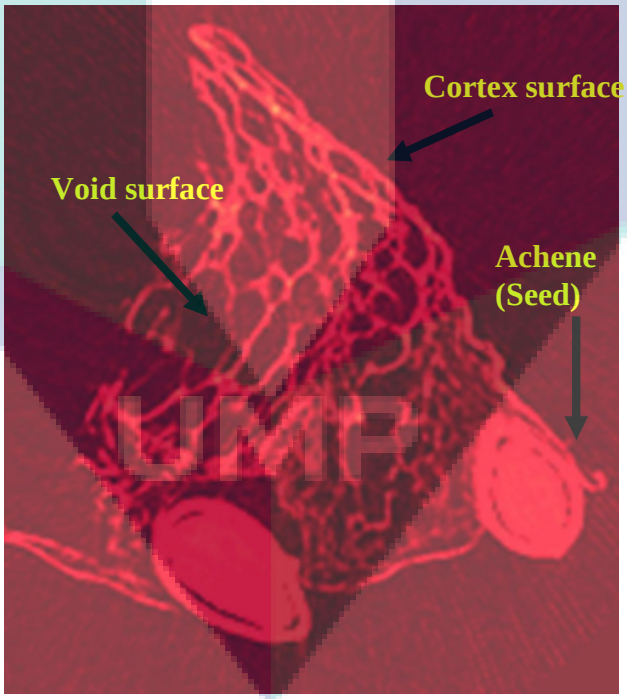

Figure 4.4 Microstructure of frozen strawberry using blast freezer after freeze-drying imaged by X-ray

Mousavi and Taghi (2007) found in Kidmose and Martens (1999) that the texture and microstructure of carrot slices with freezing had changed. They found that blast freezing resulted in low texture peak caused by major tissue damage, while cryogenic freezing gave better preservation of the native microstructure together with an increase in peak force. Tomographic image of strawberry samples after freeze drying showed larger ice crystals. Fennema et al., (1973) reported that during slow freezing, intracellular and extracellular crystallization might occur. As in these results, the cell walls and membranes also undergo mechanical stresses from the volumetric increase of the ice crystals. The freezing samples of strawberry after freeze-drying had been disrupted by this ice crystals compression. Cell wall disruption was observed by Sun and Li (2003) and showed that larger extracellular ice crystals formed during slow freezing.

Khan and Vincent (1996) observed that the mechanical damage induced by controlling the freezing rate. They found that changes in mechanical behavior of the material were directly related to the degree of cell damage from ice crystals pushing the cells apart or rupturing cell walls and thus it would produce large voids within the tissue material. Mousavi et al., (2005) showed that the voids detected by this technique could gather information on void (and thus, ice crystal) size without the experimental complexity. This method is proved to be capable of visualizing the ice crystal void structures without difficulty.

4.4 ICE CRYSTAL WIDTH SIZE FOR DIFFERENT FREEZING TECHNIQUE

Figure 4.5 shows the ice crystal average width size for two different freezing temperatures for strawberry samples, which were frozen at -20^{o}C and -80^{o}C before undergoing freeze-dried techniques. The measurements were done directly from photomicrographic images in order to provide statistically analysis. The projected area of a ice crystal was determined by segregate the perimeter of the void on a digitizing Image J and CTAN software connected to a microcomputer. The size of each void of ice crystal, defined as the diameter of a void of area equivalent to the surface projected

area, was then calculated by that software. Ice crystal size distributions were generated by grouping void sizes into 10 to 50 measurement. The average diameter or width of void area plotted in Figure 4.5 had showed the average width size distribution for two different freezing condition. These figures depicted the ice crystals of size 0.15-0.29 mm for freezing under -80^{o}C and 0.29-0.40mm for freezing samples at -20^{o}C. In this study, changes of ice crystal formation are observed quantitatively. The ice crystals size and shape were determined by the Image J and CTan software. Ice crystals formation in strawberry samples that were frozen at -80^{o}C were smaller in diameter surface area than the void measurement left in the samples that were frozen at -20^{o}C.

There is a highly significant correlation ($r = 0.59$, $p < 0.04$, $n= 50$) between each measurement taken for the samples that were frozen at -20^{o}C as compared to the samples freezing at -80^{o}C ($r = 0.26$, $p < 0.03$, $n= 50$). This high correlation suggest that the cooling rates lead to the development of a dendritic ice crystal structure that changes from slow to rapid freezing rate of the sample: this is clearly seen by the X-ray. For the slow frozen condition (-20^{o}C) of the samples, the changes in ice crystal within the same sample can clearly be seen. The ice crystals generally form in the direction of the heat flux, although some distortion is seen. However, for the fast frozen sample, there were only small dendrite or voids visible in the image; here, the freezing rate is so rapid that small visible ice crystal can be seen. This might be attributed to its high rate of freezing creating even and small ice crystals throughout the products.

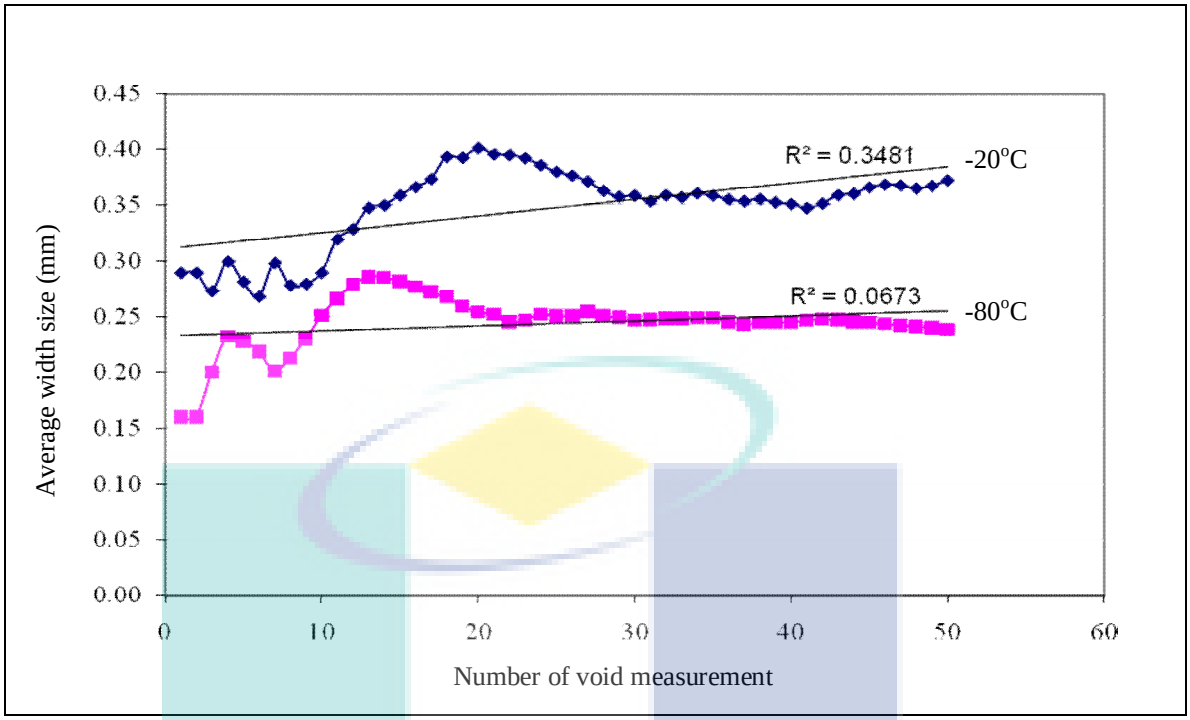

Figure 4.5: Average width size of ice crystal for different freezing temperatures

4.5 EFFECT OF COOLING DISTANCE FOR DIFFERENT FREEZING RATE

The tomographic image of strawberry samples slowly freezing at -20°C and rapidly freezing at -80°C for 7.435, 9.700 and 12.945 mm from cooling surface were shown in Figures 4.7 and 4.8. Mousavi et al., (2005) agreed that cooling rates led to the development of a dendrite ice crystal structure and the changes were from the bottom to the top of the samples could be clearly seen by the X-ray. The changes and differences of ice crystals width size could be clearly seen to produce some needle-shaped voids in the samples that were frozen at -80°C. These were attributed to its high rate of freezing and thus created very small needle-shaped ice crystals throughout the samples. While in slow freezing at -20°C, ice crystal distribution was much bigger due to the freezing rate that was applied to the samples. Other than that, it was seen in those samples the cell wall is more ruptured as compared to the samples that were rapidly freezing.

The results also compared clearly the shape of ice crystals average width size that changed from sample base to its top and thus created the different width distribution of ice crystals in the samples which is shown in Figure 4.6. The data illustrated the mean average size for ice crystal width in the samples that were frozen at -80^{o}C is highly significant; the mean average size for top of the samples were 0.30 mm ($r = 0.314$, $p < 0.07$, $n = 50$) in the middle mean was 0.24 mm ($r = 0.259$, $p < 0.02$, $n=50$) and the bottom was 0.14 mm ($r = 0.891$, $p < 0.01$, $n=50$). As seen, ice crystal width increased from the bottom to the top of the samples. These happened due to the top of the sample was far from the cooling surface and had led to larger ice crystals; while the bottom sample was nearer to the cooling surface and thus had created much smaller ice crystals throughout the samples. Mousavi et al., (2005) observed that the differences could be directly attributed to the rate of cooling which is more slowly when the samples were far from cooling surface and these had led to larger ice crystals. This study also agreed that it was possible to influence the microstructure by changing the freezing conditions and also the treated condition of the samples itself. For the samples that were nearer to cooling surface, the freezing was more rapid in the samples as compared to the other samples that were placed far from the cooling surface. The top of the samples is slowly freezing and these had lead to larger ice crystal dendrite spacing in those samples.

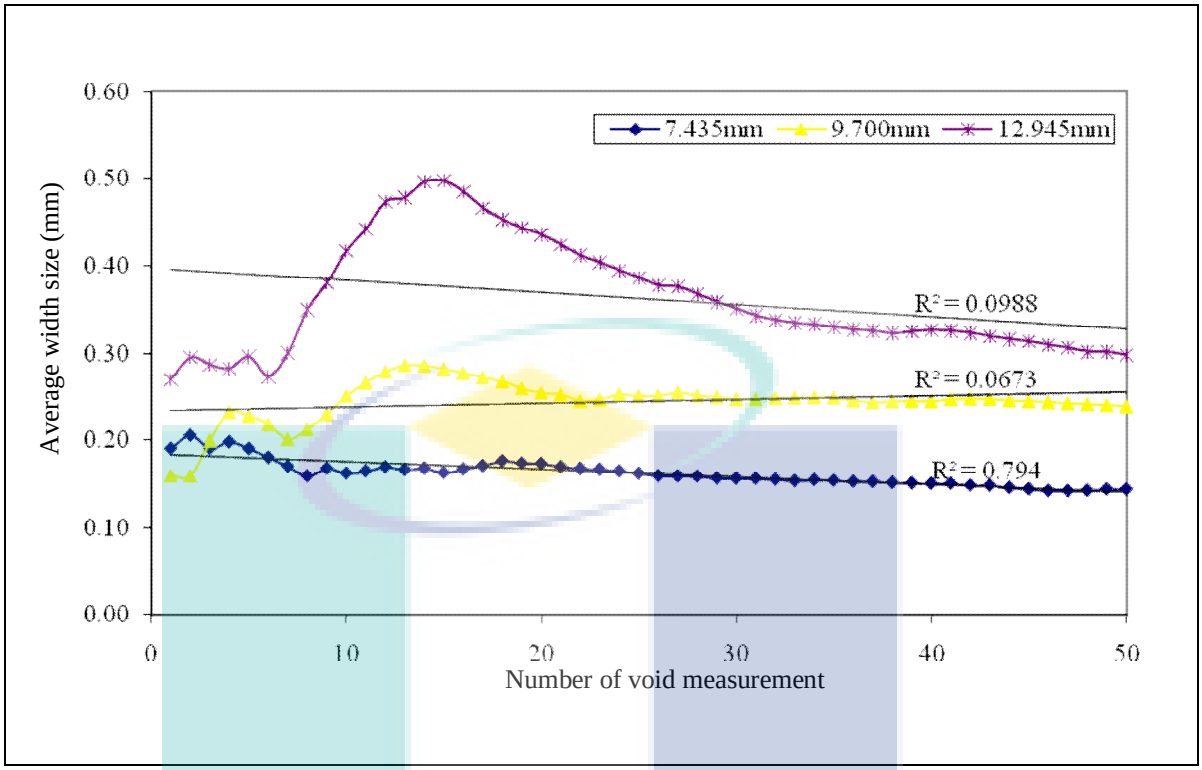

Figure 4.6: Average size of ice crystal for different cooling distance in the strawberry samples that were frozen at -80°C

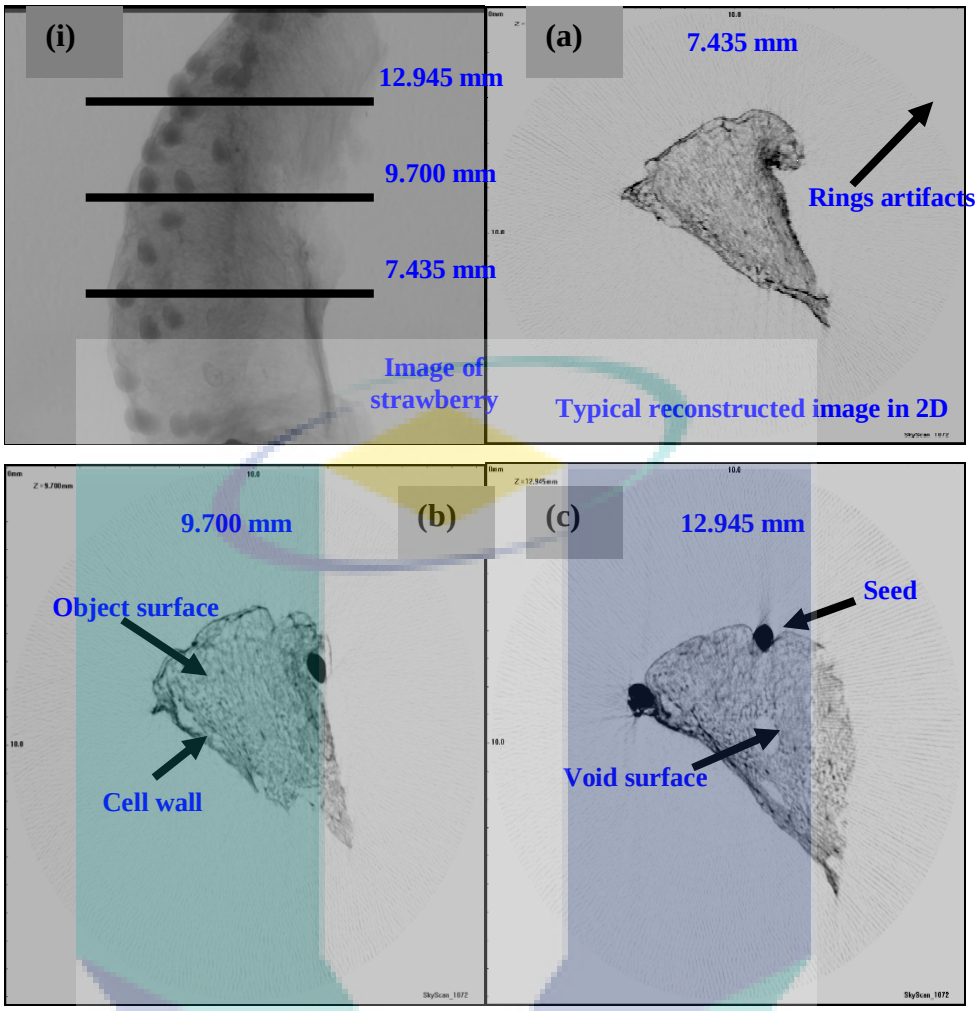

Figure 4.7: Tomographic image of freezing strawberry at -20°C: (i) typical side view of X-ray image; (a) 7.435mm from cooling surface (b) 9.700mm from cooling surface and (c) 12.945mm from cooling surface

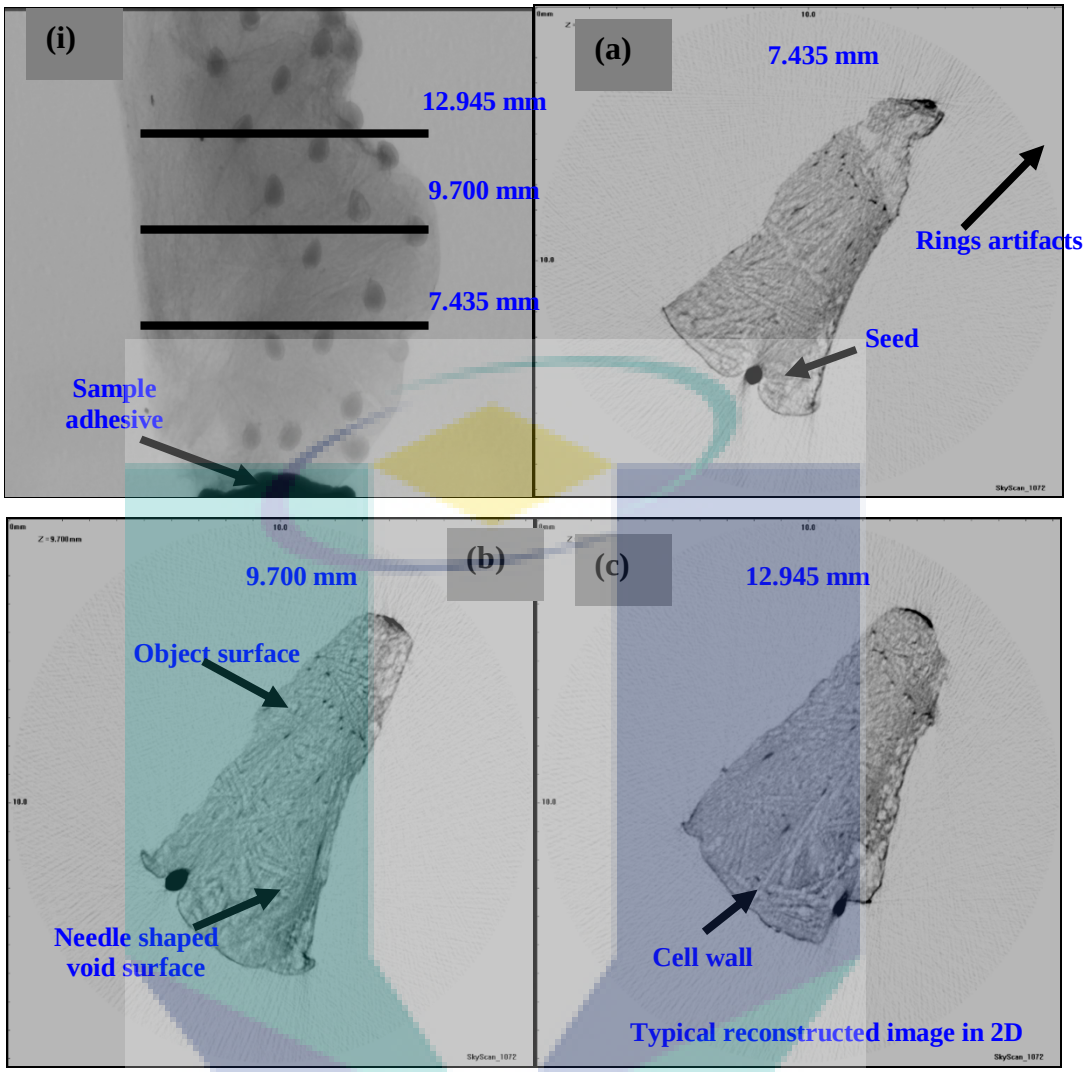

Figure 4.8: Tomographic image of freezing strawberry under -80^{o}C: (i) typical side view of X-ray image; (a) 7.435mm from cooling surface (b) 9.700mm from cooling surface and (c) 12.945mm from cooling surface

4.6 EFFECT OF THICKNESS OF THE SAMPLES ON ICE CRYSTAL SIZE DISTRIBUTION

Figure 4.9 shows the trend in thicker (15mm) sample resulted in bigger ice crystal distribution measurement compared to the thin (5mm) samples. Those samples were rapidly freezing at -80^{o}C and the trend was found to hold for the samples that had been slowly freezing at -20^{o}C. The 15mm samples was lead to bigger ice crystal formation in strawberry fruit tissues which average width size in the range of 0.17- 0.38 mm ($r = 0.578$, $p < 0.04$, $n = 50$), while 10 mm were in the range of 0.15- 0.21 mm($r = 0.568$, $p < 0.01$, $n = 50$) and 0.09 -0.24 mm($r = 0.769$, $p < 0.03$, $n = 50$) for the thickness of 5mm of the samples. The results were found to have smaller ice crystal structure and less cell wall rupture in the 5mm and 10mm samples. Freezing of different thickness of strawberry samples in different freezing temperatures and conditions produced different shapes and average width size of ice crystal. More thicker of the samples had made the cooling rates become more slower to spread over the samples and these had cause the cell wall rupture due to the formation of bigger ice crystal.

The results suggest that the samples of strawberry for this freeze-drying technique should be lesser than 5mm thickness in order to maintain the cell wall of the fruit tissue and quality of this product. The formation of small ice crystals limits the tissue destruction of these fruit that generally encountered during freezing and this could be done by having a thin piece of the samples so that it will received high rates of freezing. Product texture is better preserved at high rates of freezing. It was proven here that the rate of freezing increases when the loading density, specific surface of the product (cutting), height of the product pieces and product layer thickness decrease. These results were agreed with Maltini and Giangiacomo (1976) that had showed the freeze-drying time for intact strawberries was 30% longer than for the strawberry pieces. Le Loch (1992) also showed that the freeze-drying time of a 3 cm thick layer of common mushroom slices was 1.5 times longer than for a 1.5 cm thick layer of slices.

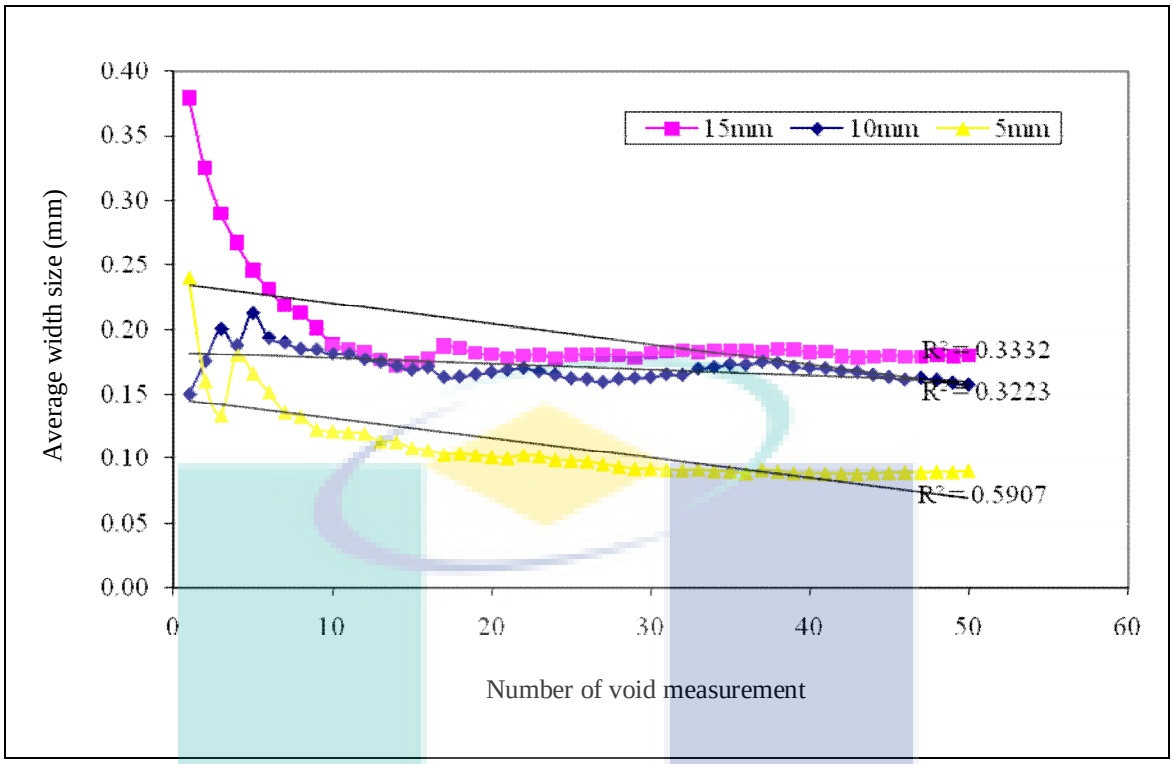

Figure 4.9: Average width size distribution of the ice crystal measurement for the different thickness of the samples that frozen under -80°C

Furthermore, the effect of rapid freezing at -80°C in various samples thickness had been evaluated. Figure 4.10 shows the width distribution of the ice crystal measurement for the 10 mm thickness of the strawberry samples which were frozen under freezing temperature at -80°C. This trend was found to hold for the other thickness of the samples that had been frozen at this freezing temperature. These figures depicted the width size vary, even though the same thickness of the samples had been frozen in the same freezing temperature. The result illustrated from 50 measurements in that sample shows an average width size of the void range were about 0.27 to 0.57 mm and the most frequently average size of the ice crystal void were about 0.32 mm. The mean for the average width size distribution in these samples was 0.27 mm and the standard deviation was 0.11 mm. The width size was differed in this sample pieces due to the cooling rates that were not uniformly distributed in this samples. The rates of cooling were much slower in the middle of the sample and this had lead to greater ice crystal distribution as compared to the top and bottom of this fruit tissues. This

condition had lead to average width size vary even though in the same thickness of the sample.

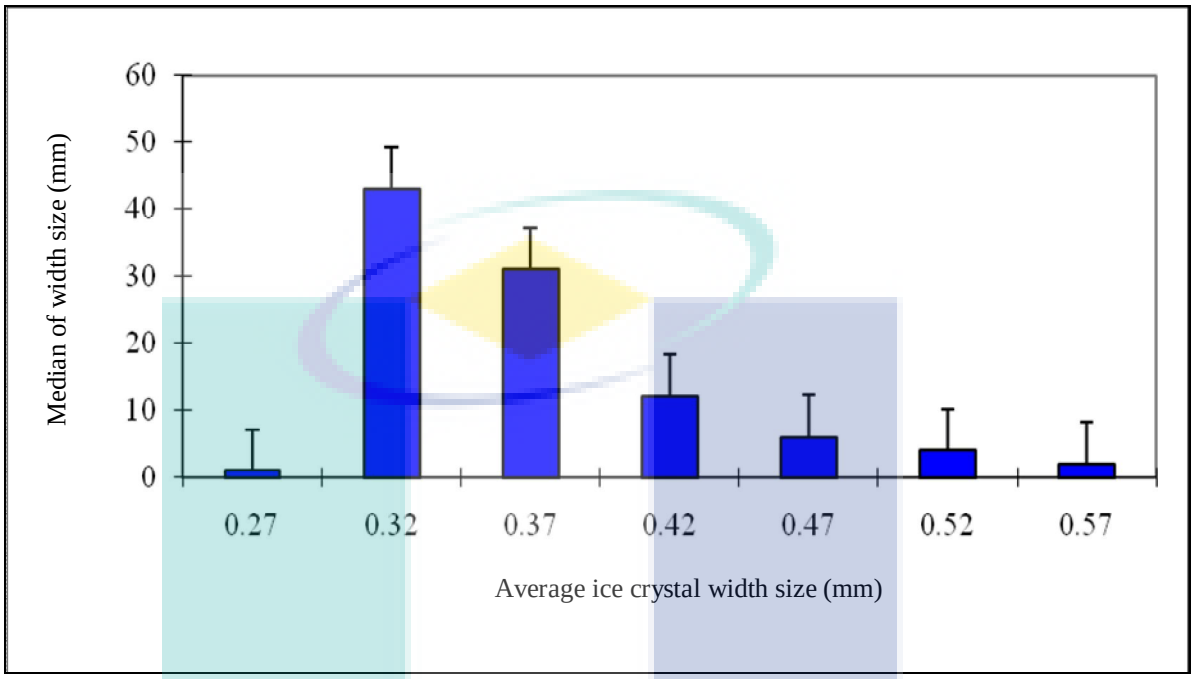

Figure 4.10: The median and standard deviation of average ice crystal width size distribution for 10mm thickness of the samples frozen at -80^{o}C

Other than that, the samples thickness also had an effect on ice crystal formation in those samples that were frozen differently under normal refrigerator condition at 4^{o}C, -20^{o}C and -80^{o}C. The trend that showed different average width size of ice crystal that was freezing at those temperatures is illustrated in Figure 4.11. The thickness of the samples was 5 mm and the results were evaluated for three samples that had same thickness which were treated at different temperature. The figure depicts that the average width size in 5 mm samples treated either at 4^{o}C, -20^{o}C or -80^{o}C was highly significant which is in the range of 0.06- 0.19 mm ($r = 0.176$, $p < 0.06$, $n = 50$) only. Even though the processing condition was different, but, in this extent of studies, the small or thin pieces of strawberry had lead to smaller ice crystal size in those samples. The cooling rate was much rapid in the thin samples as compared with thicker samples. The findings were agreed with Hammami and Rene (1997) that the use of thick layer was led to long processing times as compared to those generally encountered at the pilot

scale which were in thin or monolayer, low quantities and very low loading densities of the products.

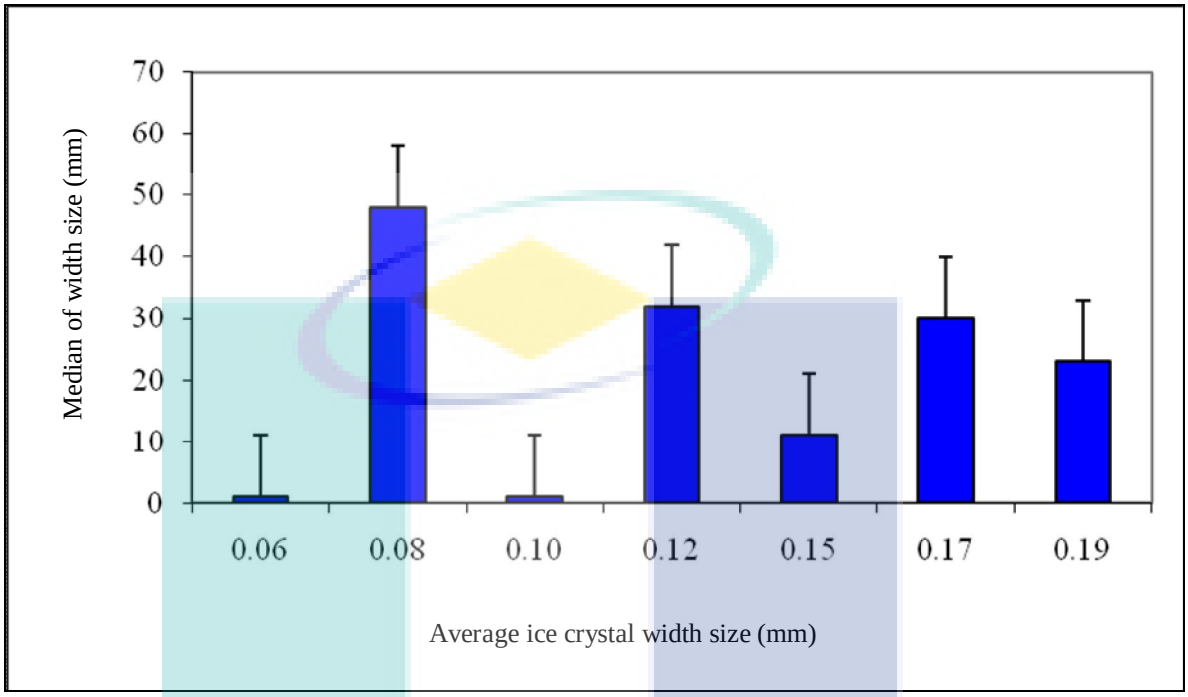

Figure 4.11: The median and standard deviation of average ice crystal width size distribution for 5mm thickness of the sample frozen at different cooling rates (4°C, -20°C and -80°C)

4.7 MICRO STRUCTURAL INFLUENCE ON ICE CRYSTAL SHAPE

Mousavi et al., (2007) observed using x-ray micro tomography that cheese and potato did not produce any difference in ice crystal shape and structure in the freezing conditions due to its similar microstructure in any position. The results in this study were not established with Mousavi et al., (2007). At each different thickness, the ice crystal width distributions of the samples were in different range and shape along with the different axial position, were also not the same to each other. The variation of images was due to the differences in the number of ice crystals width size at different cooling rates. The result were depicts in Figure 4.12 showed that the average ice crystal width size were much larger at cooling rates -20°C which were in the range of 0.15-0.21 mm ($r = 0.568$, $p < 0.01$, $n=50$). The average ice crystal width sizes were smaller in the range of 0.06-0.08 mm ($r = 0.825$, $p < 0.005$, $n = 50$) at rapid freezing -80°C. However,

the average width of ice crystal were uniformly distributed and medium in size which is in the range of 0.11-0.17 mm ($r = 0.855$, $p < 0.02$, $n = 50$) for the samples that was cooled at 4°C as compared to the samples that were frozen at -20°C and -80°C. The most likely reason to these differences measurement was on how the images were treated and not the differences between the ice crystal widths seen by this technique.

The images shown in Figures 4.13, 4.14 and 4.15 were the different shapes of ice crystals produced by three different cooling rates condition (4, -20 and -80°C) for different thicknesses (5mm, 10mm, 15mm) of the samples. They demonstrated similar shapes of void for each freezing samples but the size of the void was different neither in each axial position nor the thickness itself. The relationship between microstructure and ice crystals was clearer when these ice crystals structure formed in different axial position and thickness of the strawberry. Ice crystals in the thicker samples of the strawberry were larger than the thin of the samples. The trend was found to hold in any cooling rates applicable to those samples. It was clear here that the size or thickness and direction or position of the samples mostly affected the size of the ice crystal.

However the shape of the ice crystal and structure in different cooling rates condition were also significantly different even though the strawberry samples possessed similar microstructure at either direction. Tomographic images of ice crystal distributions for strawberry samples at different cooling rates in different thickness were shown in Figures 4.13, 4.14 and 4.15. Tomographic images showed an increase in the void measurement of the width as the cooling rates were decreased. Thus, it should be concluded that the x-ray technique able to visualize the ice crystals information in the frozen strawberry. Woinet et al., (1998) had agreed and suggested that the x-ray micro-computed tomography is accurate for ice crystal measurement and this method had the capability in measuring the effect of different cooling rates conditions of the process.

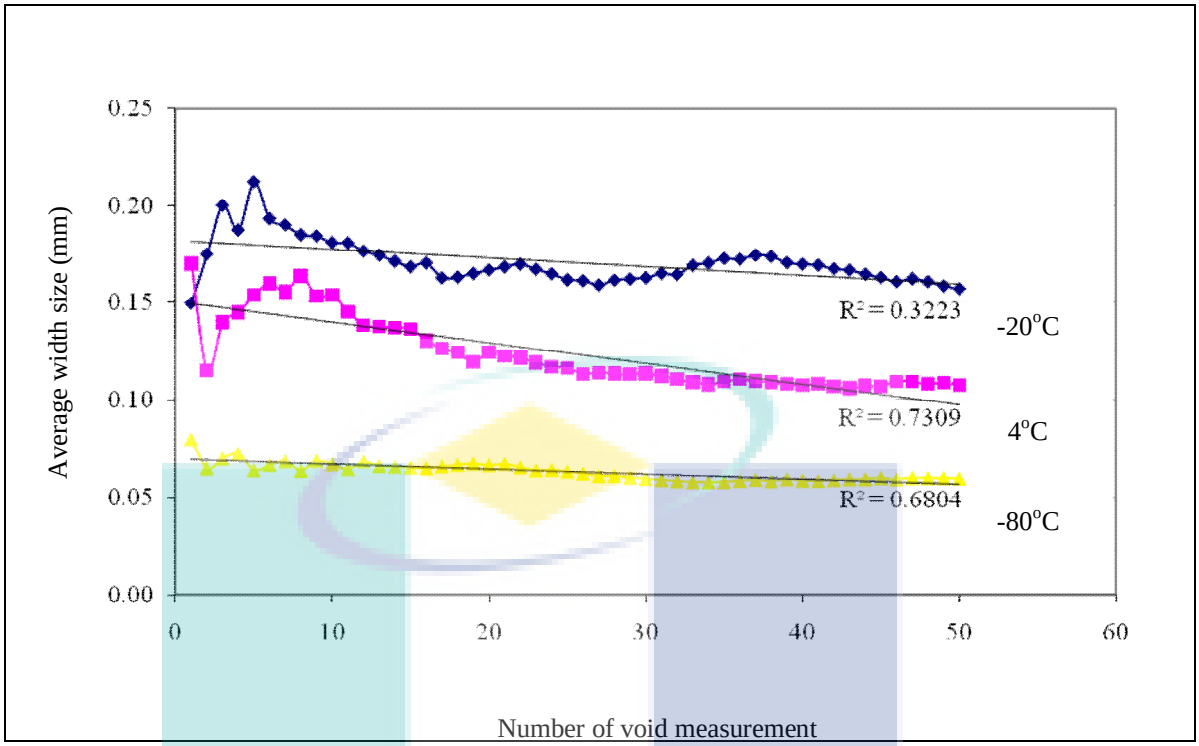

Figure 4.12: Average width size distribution of the ice crystal measurement for different cooling rates (4, -20 and -80°C) in 5mm thickness of the samples

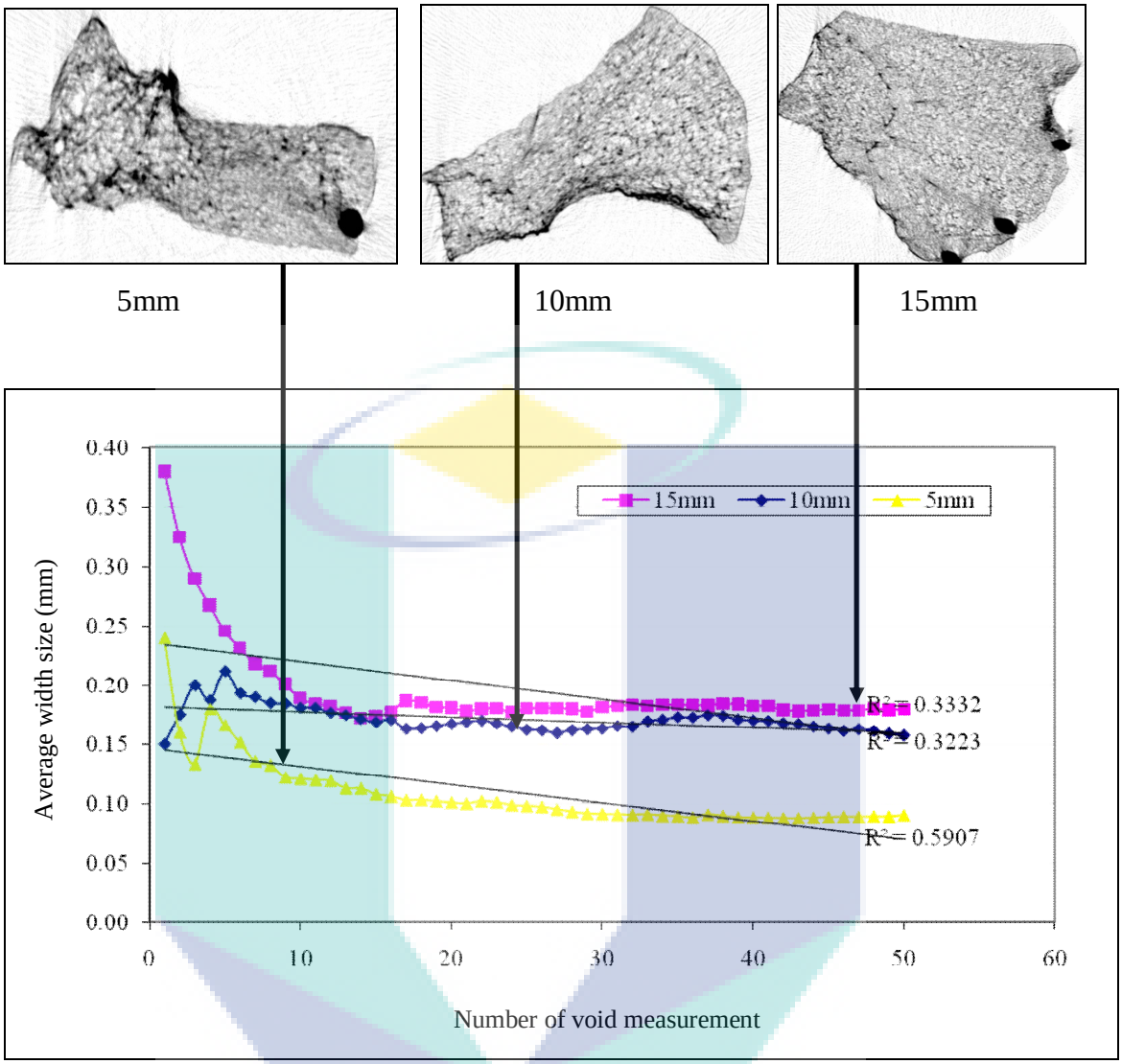

Figure 4.13: Average width size distribution of the ice crystal measurement and tomographic image of microstructure for different thickness of freeze-dried strawberry samples freezing at -80°C

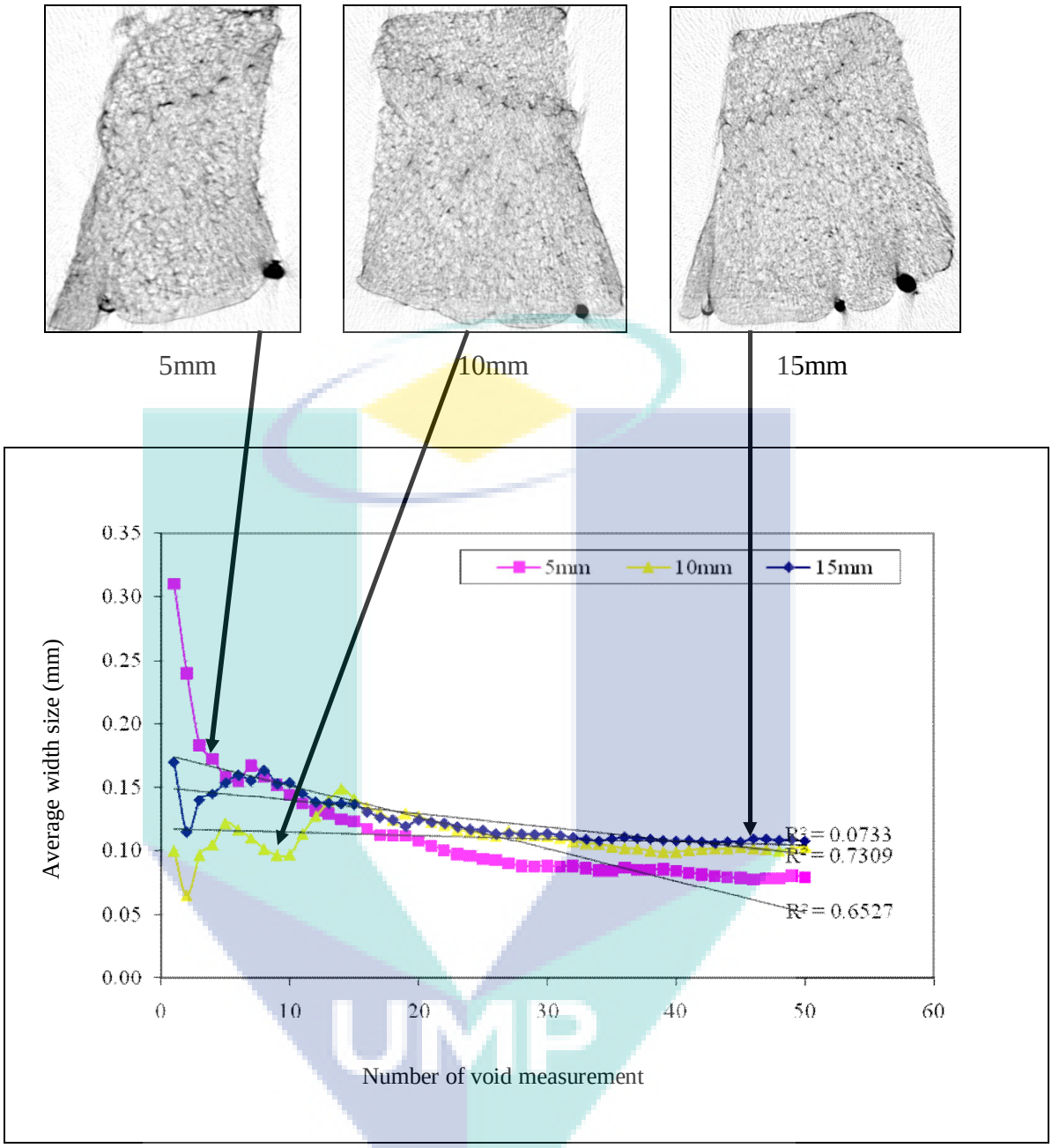

Figure 4.14: Average width size distribution of the ice crystal measurement and tomographic image of microstructure for different thickness of freeze-dried strawberry samples freezing at 4°C

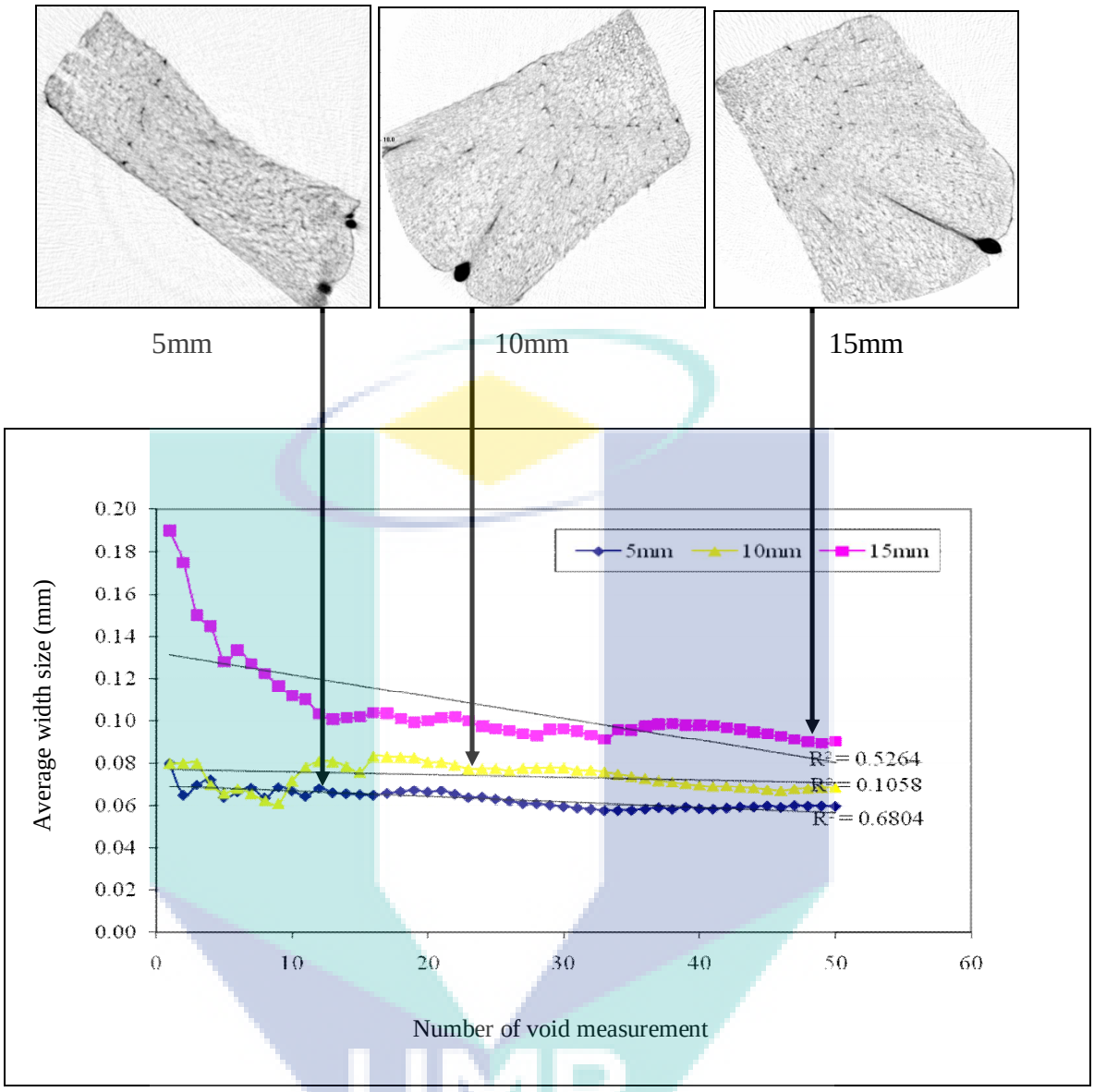

Figure 4.15: Average width size distribution of the ice crystal measurement and tomographic image of microstructure for different thickness of freeze-dried strawberry samples freezing at -20°C

4.8 ICE CRYSTAL VALIDATION PROCEDURE

The findings in 4.3 showed that there was an increase in the porosity of freeze-dried strawberry as opposed to the fresh material. However, it was not clear if the needle-shaped voids seen in x-ray scanning of freeze-dried materials corresponded to the space left by ice crystals or whether they were artifacts. To validate whether the dendrite spacing seen by x-ray method is the space that was originally filled by ice crystals, the x-ray images were taken from different axial locations of the strawberry and jackfruit samples. To analyze the x-ray image at different axial positions from freezing plate, around 10 to 20 slices obtained by the related Sky scan software (2D image obtained after multiple-slicing of shadow image at right angle to heat flux) were randomly chosen from related slices of the samples for each axial position. Slices were taken from 3 parts of the sample (i) sample base, from 450 to 1500 µm from freezing plate; (ii) sample center, from 2500 µm to 3500 µm from freezing plate, and (iii) sample top, from 4500 µm to 5500 µm from the freezing plate.

Strawberry samples types of images were processed by the corresponding image processing system. At least 50 measurements of ice crystal width were obtained from micrographs for each technique at each condition. Furthermore, a number of 3D parameters can be obtained from 3D model of strawberry samples which is including void cell volume fraction, cell surface to volume ratio, and cell wall thickness. A detailed distribution from 3D model of strawberry samples were not in the scope of this investigations, so the findings were just limited to image visualisation of this 3D model of the samples that was clearly shown in Figure 4.16. While for jackfruit samples, it was compared based on visualisation of the image in various axial position and the microstructure determined by x-ray methods for freeze-dried jackfruit and strawberry using the unidirectional rapid freezing method.

Figures 4.16 and 4.17 compared the microstructures determined by x-ray methods for freeze-dried strawberry and jackfruit samples. The microstructures of jackfruits and strawberries samples were seen to have marked differences and ice crystal–related pore structures could be seen in either method for both sample. Further

studies are still needed in term of works and observations in details for the microstructure study in freeze-dried jackfruit samples. In this study, the result for jackfruits samples was just for validation procedure of ice crystals formation in frozen fruits and it was limited to image visualisation only. A detail distrubution of microstructure studies for jackfruits samples were not in the scope of this study and future reccomendation were highly suggested for further analysis of these samples. These samples had been used to validate that the x-ray micro-CT was successful in scanning ice crystal formation in fruit tissues. The difference between the density of void in the cell and that of the surrounding fruit materials led to a good contrast in the reconstructed images, as shown in Figure 4.16 and 4.17.

Previous studied by Mousavi et al., (2005) had validate the dendrite spacing seen by x-ray method is the space that was originally filled by ice crystals using SEM and x-ray images that were taken from different axial locations of the three mycoprotein products of paste, dough, and steamed dough were analyzed. Thus, it should be concluded that the x-ray technique can detect ice crystal information in the material. The information presented here clearly shows the capability of the x-ray technique to observe ice crystal structures inside solids. This is not possible by other ice crystal measurement methods at present. Therefore, the methods could be helpful in obtaining information about ice crystals in other food materials as well as in other industrial applications.

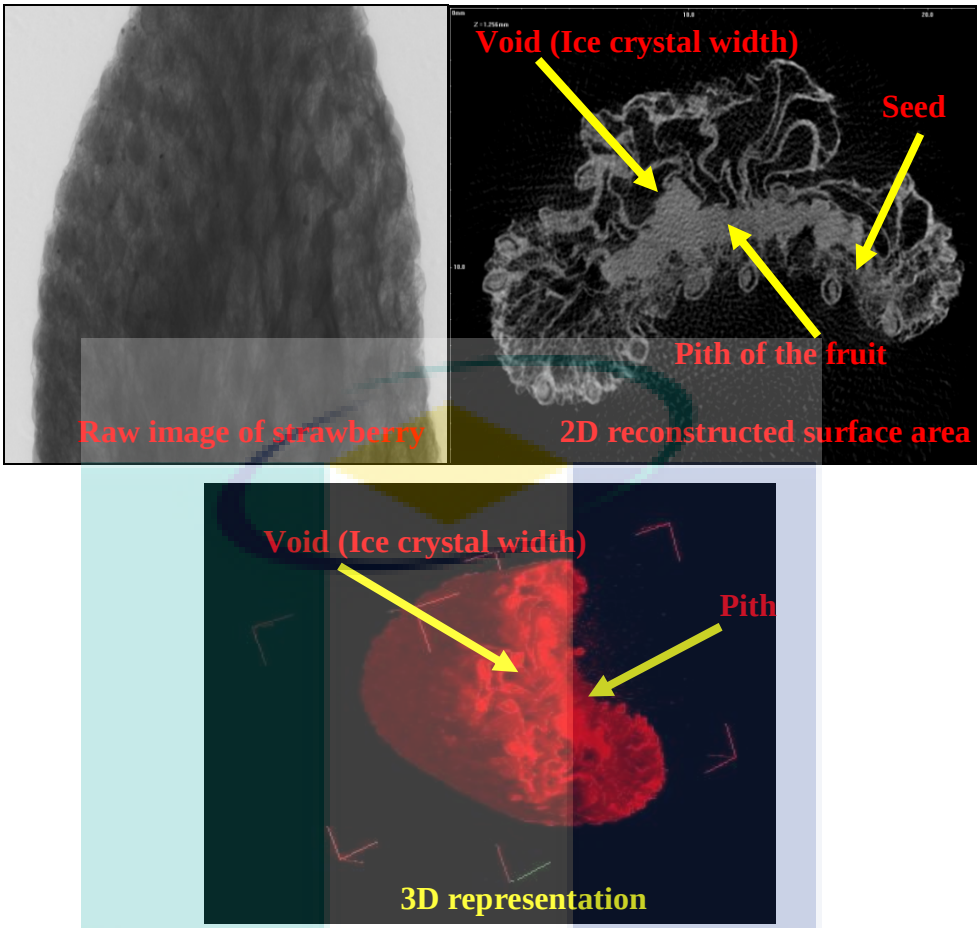

Figure 4.16: Image visualization of freeze-dried strawberry in 2D and 3D view

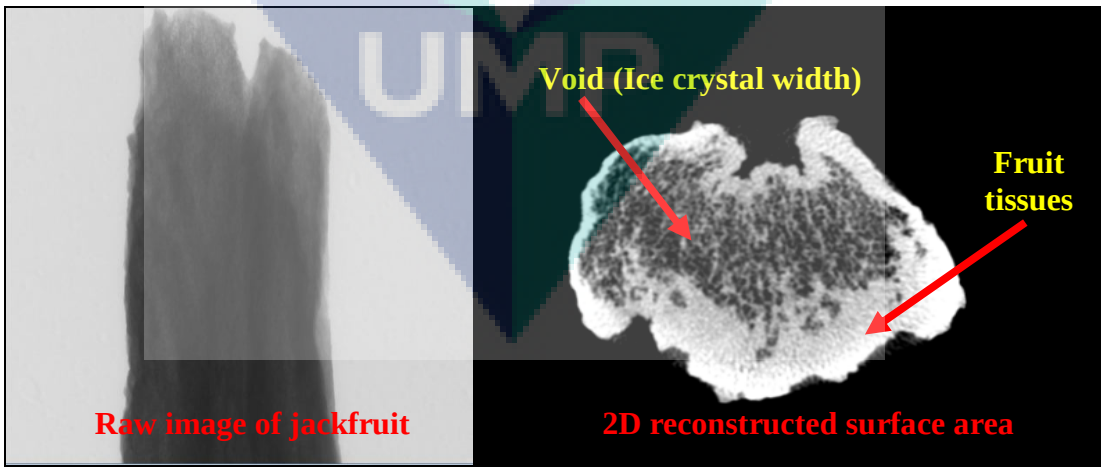

Figure 4.17: Image visualization of freeze-dried jackfruit in 2D view

4.9 COMPARISON OF DRYING METHOD

The previous part of this study showed the microstructural changes due to the influence of processing parameters (freezing rate and thickness of the sample), however throughout this study, the freeze-drying method had been compared with the air-drying techniques by using microwave temperature which is at 60^{o}C. In this part of the study, the investigation is being limited to microstructural image analysis using x-ray micro-computed tomography on strawberry samples. The result shown in figure 4.18 below is the image of microstructural changes of strawberies sample using air-drying technique. The image presented here clearly shows the capability of the x-ray technique to observe ice crystal structures inside air-dried strawberries. The results had been compared with the freeze-dried sample of the strawberry in Figure 4.19. It is shown that, the cell wall ruptured bigger in air-dried sample as the heating already affected the material of the samples. The colour of the samples changed to brownish and the aromatic compound of strawberries decreased. The losses of fruit proteins had resulted in this browning reactions. In conventional thermal heating, energy is transferred to the material through convection, conduction and radiation of heat from the surface of the material (Chih et al., 2002). In contrast, the study was agreed with Abdurahman et al., (2006), that the microwave energy is delivered directly to the material through molecular interaction with the electromagnetic field and this microwaves can penetrate materials and deposit energy. Other than that they also found that the heat can be generated throughout the volume of the material.

Furthermore the bigger cell wall rupture in air-dried strawberry samples were shown in Figure 4.18 and this was due to the rapid and uniform heating in this conventional air-drying technique. The findings was agreed with Thostenson et al., (1999) that found the transfer of energy do not rely on diffusion of heat from the surfaces and it is possible to achieve rapid and uniform heating of thick samples. However, heating had affect the molecular structure of the fruits and destroyed some of its nutrients (losses of micronutrient such as ascorbic acid). In order to prevent this happen to fruits, the freeze-drying had been proven one of the novel drying technique for improving the product quality. As shown in Figure 4.19, the cell wall is well

retained and more smaller ice crystal had been found in this samples. These samples were found to hold its original flavour, colour and nutrients. Besides that, these technique had made the fruits structure more better conserved and will be easily to rehyrdate when in need to use it as original fruits.

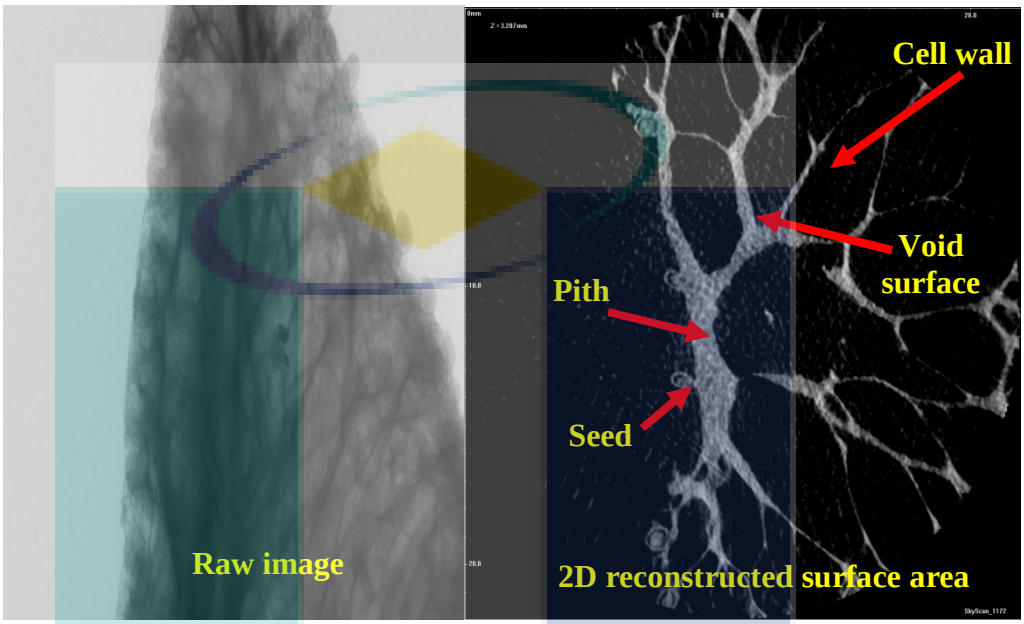

Figure 4.18: Image visualization of air-dried strawberry in 2D view

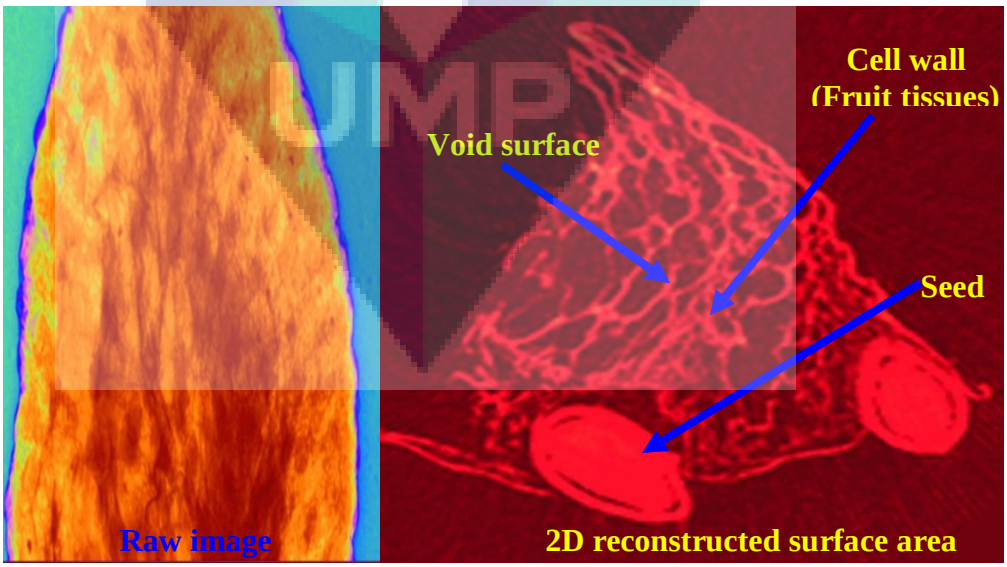

Figure 4.19: Image visualization of freeze-dried strawberry in 2D view

4.10 COMPARISON OF XMT OVER MRI IMAGES ANALYSIS

The image analysis of dried strawberries using MRI techniques was previously obeserved by Otero et al., 2009 and it had been compared with the images in Figure 4.19 studied by using XMT techniques throughout this investigation. The differences of images visualised by both techniques proved that XMT techniques employed, offered the opportunity of studying the whole product without any preparative manipulation which could mask the ice crystal effects. The results obtained should be useful to understand how and why ice crystal causes damage to fruit tissues and, therefore, to design optimized freezing and freeze-dried treatments which minimize this damage.

Fruits and vegetables are particularly interesting food products for freezing processing due to their delicate sensorial and nutritional characteristics which are especially thermolabile. Nowadays consumers are demanding for fresh, natural and healthy food with an extended shelf life which freezing processing can represent, in some cases, an appropriate solution. But the effects of ice crystal formation on vegetal products are variable and dependent not only on the process variables (pressure level, holding time and temperature) but also on the composition and form of the food treated (whole product, slices, puree or juice) (Otero et al., 2009).

Ice crystal formation can significantly alter vegetal tissues and induce changes in their structure and texture which, along with taste, are the most important sensorial attributes, for consumers' acceptability. For this reason, most of the commercialized processed products of plant origin are in the form of puree or juice (guacamole, fruit jams and juices). Most textural changes produced by ice crystals are due to the physical disruption of the cells, but, ice crystal also enhances reactions during storage. Liquid included in vegetal tissues has a greater compressibility than air and solid components. When freezing is used, a rapid expansion of this ice crystal causes extensive damage on the cell structure. Moreover, changes occur on the membrane permeability which enable the movement of water and metabolites from the inside to the outside of the cell. Substrates, ions and enzymes, which are located in different compartments, can be

liberated during the treatment and interacted with each other during storage, through enzymatic and non-enzymatic reactions (Otero et al., 2009).

To visualize damage produced in plant tissues, several authors had employed different techniques of microscopy and they had found how pressurized fruits and vegetables showed a loose and irregular cell distribution due to cell–cell debonding, losses of turgor, cell conformation changes and cell wall disruption (Otero et al., 2009; Butz et al., 1994; Fuchigami et al., 1996; Prestamo et al., 1998; Tangwongchai et al., 2000 and Trejo Araya et al., 2007). Damage induced by pressure depended on the product processed and usually increased as pressure and processing time increased (Otero et al., 2009; Basak et al., 1998 and Prestamo et al., 1998). Soft products like spinach, strawberries or tomatoes are particularly sensible to pressure and micrographs of these products showed extensive damage in the tissues when they were treated at pressures higher than 300 MPa. At lower pressures, damage detected by microscopy seemed to be considerably minor (Otero et al., 2009; Butz et al., 1994; Tangwongchai et al., 2000 and Marigheto et al., 2004). But, at these relatively low pressures, the increased cell permeability could produce an extensive redistribution of water in the tissues, which is not easily monitored by traditional techniques but it indisputably affects the food quality.

In this sense, X-ray Micro-computed Tomography (XMT) is a particularly promising technique because it offers the opportunity of studying foods in their wholeness, in a non-invasive and non-destructive way, without any preparative manipulation which could alter, among others, the real distribution of water in the sample over the magnetic resonance imaging (MRI) technique (Otero et al., 2009; Chen et al., 1989 and Ruan et al., 2001). This XMT technique is especially interesting because it allows the direct study of the product and it avoids the use of extraction and separation methods which may interfere with its biochemistry. Strawberry was selected because it is a soft and highly perishable fruit with very short life span (not more than 4 to 5 days in refrigeration conditions). Previously, MRI techniques have been successfully used in the literature for studying the effect of natural ageing, ripening, mechanical damage, pathogen infection and/or processing in different fruits and

vegetables (Otero et al., 2009; Hall et al., 1998; Clark et al., 1997; Gil et al., 2000 and Ribo et al., 2004) but, to this extent of knowledge, little is known about the application of that technique in observing the crystallization formation in fruit tissues. Shaarani et al., (2006) also suggested in previous study that the future investigation should be done using the faster version of image processing protocols in order to measure the changes in food structures. Based on the obeservation details throughout this study, it was proven that microstructure changes could be seen clearly by using this XMT techniques over the other microscopic image analysis.

The work has shown that XMT is a useful tool for the study of microstruture of fruits materials especially in strawberry. The technique has significant benefits for the design, analysis, and processing science of fruit products. Such an advance in ice crystal fruit measurement will also undoubtedly open up new horizons for the development of mathematical and computational models that link product microstructure to the product mechanical properties and rheology in the future.

4.11 APPEARANCE

The results in Figures 4.20 and 4.21 show that the phenomenon of strawberry and jackfruit shrinkage correlated primarily due to the operating pressure. The findings showed in this study was agreed with those previous study done by Hammami and Rene (1997), Rene et al., (1993), Genin et al., (1995) and Cosio (1997) that the appearance quality of strawberry samples could be preserved better in freeze-drying technique that used low working pressure. In this study the vacuum condition was at 0.1 Torr and it had helped in preserved the appearance of strawberry and jackfruit sample. The freezed-dried strawberry fruits was significantly similar to fresh fruits as compared with the air-dried strawberry fruits (poor and considerable shrivelled). While for jackfruit samples, the comparison of appearnace had been done to the freeze-dried and the fresh samples. The results visualised that both freeze-dried and fresh sample were similar to each other. The appearance of those freeze-dried fruits products was assessed visually in comparison with a reference which is the fresh strawberry and jackfruit itself. Special attention was paid to shrinkage problem and jackfruit cell wall is less shrinkage as

compared to strawberry fruit tissue. This was results due to the hard fruit tissue of the jackfruit had made the fruits appearance is much better than the freeze- dried strawberry . However, the freeze-dried strawberry appearnace is much better and more attratctive as compared with the air-dried of the strawberry samples. The heating in air-drying technique had effect on the appearence of this product which is become more brownish and more shrinkable.

Previous study by Hammami and Rene (1997) had proven that the working pressure must be lower than 30 to 40 Pa in order to avoid strawberry shrinkage. Nevertheless, the working pressure could be increased up to 50 to 60 Pa without excessive shringkage of freeze-dried strawberries due to its intricate polymetric structure. Thus, to avoid the shrinkage phenomenon during freeze-drying, the temperature of the frozen core must be lower than the initial melting temperature as illustrated in Figure 4.22. Throughout this study, the initial melting temperature for strawberry was at -34^{o}C and the temperature of the frozen condition were at -20^{o}C and -80^{o}C. For the samples that were under -20^{o}C, the strawberry is more shrinkage as compared to the samples that were rapidly freezing at -80^{o}C. This explains why vegetables or milk, which present an initial melting temperature of about -15°C to -5°C (the corresponding partial pressures of water vapour are in the range of 200 - 400 Pa), could be freeze dried even at 200 Pa (RenC et al., 1993 and Genin et al., 1995) while the fruits could be freeze-dried only at pressures lower than 100 Pa. This value corresponds to a temperature of -20°C and is in the range of initial melting points for fruits.

Maltini and Giangiacomo (1976) showed that bananas and strawberries were perfectly rehydrated only if the freeze-drying pressure was lower than 80 Pa. Cosio (1997) also showed that the shrinkage phenomenon existed during the freeze-drying of banana slices at a working pressure of 200 Pa, but never appeared at working pressures lower than 50 Pa. The findings in this study also suggested that, the slow freezing rate also had affected the appearnce of the fruits which is more shrinkable and slowly to be rehydrated as compared to the samples that were rapidly freezing (less shrinkage and easily rehydrated). The main effect in those samples was the formation of ice crystal that had ruptured the cell wall of the fruit samples.

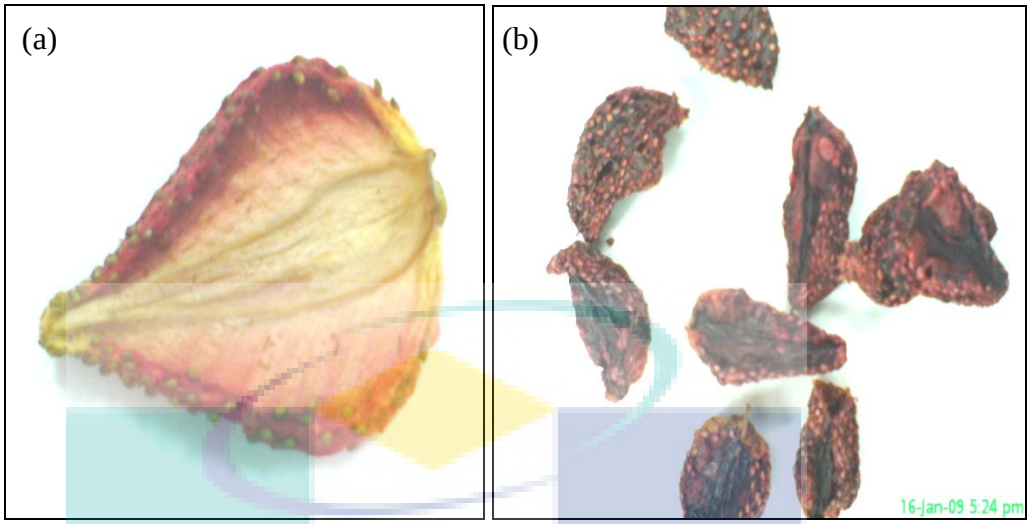

Figure 4.20: Image of strawberry shrinkage after (a) freeze-dried and (b) air-dried techniques

Figure 4.21: Image of jackfruit shrinkage after freeze-dried techniques

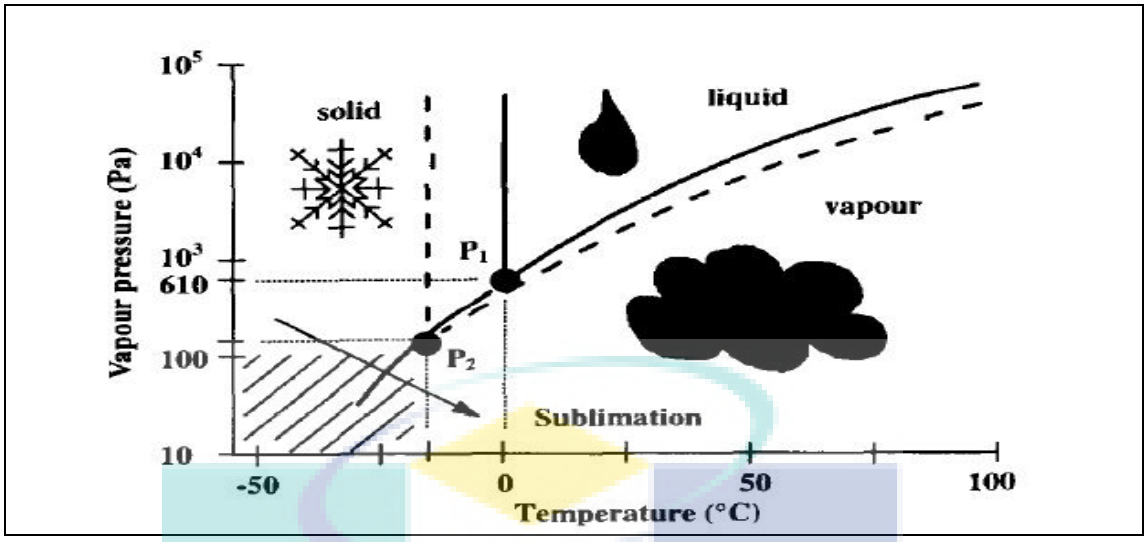

Figure 4.22: Phase diagram for water and aqueous solutions (P1: Triple point of pure water; P2: Triple point or eutectic point of an aqeous solution)

Source: Hammami (1997)

4.12 REHYDRATION RATIO

The analysis of the rehydration results showed that this criterion was independent of both operating pressure and heating plate temperature. The Fisher test probability is very high (P(F) > 0.50) and the determination coefficient was very low (R2 ~0.50). The same tendency had been observed for the rehydration of freeze-dried bananas. It was to be noted that the values of the rehydration ratio were not significantly different. These values range from 22% to 32% and were attained after soaking in heated water (50°C) for 5-20 min (Hammami et al, 1997 and Cosio, 1997). The rehydration capacity of the freeze-dried products obtained in this work was agreed to be closed to other published results. Previous study reported that the rehydration ratio of strawberries (frozen at -25°C) was about 50% for warm water soaking times from 0.5 to 5 min (Hammami et al., 1997; Carballido et al., 1970 and Simatos et al., 1974). Besides the thickness of the product, its variety (origin and degree of ripeness) and dry matter content, differences in the rehydration ratio could be related to the initial freezing. As previously mentioned, ice crystal size is governed by the freezing step (freezing rate and final temperature) and is the main parameter governing the rehydration stage. The

influence of the freezing rate and thus ice crystal size on the rehydration of freeze dried products had been extensively studied: Smithies (1962) showed that pieces of meat frozen in an acetone-dry ice mixture rehydrated much more slowly than those frozen at -20°C (Hammami et al., 1997).

The same tendency was observed for other foodstuffs such as asparagus (Hammami et al., 1997) prawns (Hammami et al., 1997 and Goldblith et al., 1964) peaches, apples and apricots (Hammami et al., 1997 and Lee et al., 1967) strawberries (Hammami et al., 1997 and Baumunk et al., 1969) bananas (Hammami et al., 1997; Maia et al., 1970, and Maltini and Giangiacomo, 1976) raspberries (Hammami et al., 1997 and Medas, 1971) carrots (Hammami et al., 1997 and Longan, 1973) passion fruit juice (Hammami et al., 1997 and Cal-Vidal et al., 1985) and onions (Hammami et al., 1997 and Genin et al., 1995).

4.13 COLOUR

The visual appreciation of strawberries freeze-dried under different processing conditions showed a slightly pronounced red and yellow for freeze dried jackfruits compared to the fresh product, but this vanished after rehydration. The increased redness of freeze-dried strawberries and yellowness of freeze-dried jackfruits led to an increase (12%) of a* and a decrease (10%) of b*. Nevertheless, these chromatic coordinate changes were comparable to frozen ones, but were more marked. Indeed, the colour of fresh and dry fruits differ as water affected the appearance.

Thus, the red and yellow colour increase could be attributed to both the freezing step and the water reduction effects. Indeed, this colour change had consistently been observed in strawberries and jackfruits placed both at the top and at bottom of the layer. This phenomenon was also reported previously for strawberries freeze-dried at 25°C and 15 Pa (Hammami et al.,1997 and Carballido et al., 1970). Previous study had also shown that the red colour of strawberry was reinforced by freezing (Hammami et al.,1997 and Wrolstad et al., 1970). This phenomenon was due to the modification of the form of one of the main strawberry dyes, pelargonidine-3-glucoside. The same

phenomenon was observed for uncooked beef meat: the sample frozen at a low freezing rate had a more pronounced redness than the one frozen at higher freezing rates (Hammami et al.,1997 and Bengstsson et al., 1968).

The more pronounced redness of freeze-dried strawberries could also be explained by a concentration effect of the red pigments (anthocyans) in the dried product. The red colour of the Pajaro strawberry easily darkens (Hammami et al.,1997 and Roudeillac et al., 1987). Similarly, the pulp of strawberries freeze-dried at 64°C and 173 Pa presented a slight browning. At 60°C, the skin and the pulp of air-dried strawberries presented considerable browning. Strawberry is consistently more sensitive to this colour change than the jackfruit. Differences in composition, particularly sugars, may explain this phenomenon.

To avoid variations due to the product rehydration (dissolution of red and yellow dyes), colour measurements were performed directly on the freeze-dried strawberries and jackfruits. Statistical processing of the results was carried out to determine the influence of processing conditions parameters, by comparison with the colour of the fresh product, with the following variables in equations 4.1 and 4.2 (Hammami et al.,1997) :

$$(1 - a_f^*/a_i^*) \quad (4.1)$$

$$(1 - b_f^*/b_i^*) \quad (4.2)$$

The indices f and i correspond to the final (freeze-dried) and initial (fresh) strawberry and jackfruit. When necessary, indices s and j had been added to differentiate products. Since the variation coefficient of calorimetric measurements of fresh strawberries was about 10%, this value was used as the limit beyond which the difference was considered as statistically significant. The statistical colour which resulted from the strawberry and jackfruit dried in different processing conditions, showed that the colour parameters were dependent on the heating plate temperature, species and sizes. It should be noted that the colour of strawberry and jackfruit situated at the top of the layer was not affected by the processing conditions. However, to obtain

a homogeneous colour batch of freeze-dried strawberry and jackfruit, same species and sizes had to be used and situated at the bottom of the layer.

Fruit colour was a major determinant of quality in red berry fruits or other perishable fruits like jackfruits and their products and it was due to the presence of anthocyanins, a group of water-soluble pigments with antioxidant properties. The main anthocyanins present in strawberry are pelargonidin-3-glucoside and cyanindin-3-glucoside (Ankit et al., 2009 and Zabetakis et al., 2000) respectively and recent work had suggested involvement of anthocyanins in various health benefits and cancer prevention (Ankit et al., 2009 and Zhang et al., 2004). Despite the fact that high pressure processing has been used in Japan commercially and other countries for some years, the effect of high pressure processing on antioxidant activity, and different antioxidant groups (anthocyanins, phenols, ascorbic acid) has not been extensively studied in blackberry and strawberry purees (Ankit et al., 2009).

4.14 NUTRITIONAL CHANGES DURING PROCESSING

The freeze-dried process used to freeze-dried strawberry and jackfruit product leads to some changes in micronutrients content. The most significant changes that occured were when strawberry had become a frozen product and the amount of the ascorbic acid content decreased. As shown in Table 4.4, freezing had changed the caloric content of the strawberries as well as the amount of sugar and total carbohydrate (analytical data done by SIRIM Qas International Sdn. Bhd.) found in the product. Frozen processing does not have an impact on sodium, fiber, Vitamin A, or Calcium that were analytically done SIRIM Qas International Sdn.Bhd. and had been compared with previous work by Christina, 2005.

Table 4.4: Nutrients information (± standard deviation) for fresh, and frozen strawberries

Nutrient (221g)	Fresh (Current Study)	Fresh (Christina, 2005)	Frozen Unsweetened (Current study)	Frozen Unsweetened (Christina, 2005)
Calories	**65** (± 18.35)	71	**71** (± 24.35)	77
Sodium	**3 mg** (± 0.93)	3 mg	**3 mg** (± 0.95)	4 mg
Total Carbohydrate	**12 g** (± 3.72)	17 g	**15 g** (± 4.65)	20 g
Dietary Fiber	**4 g** (± 1.03)	4g	**5 g** (± 1.05)	5 g
Sugars	**8 g** (± 2.47)	10g	**8 g** (± 2.43)	10 g
Vitamin A	**0 %** (± 0.00)	0 %	**0 %** (± 0.00)	2 %
Vitamin C	**149 %** (± 46.19)	217%	**132 %** (± 40.92)	152 %
Calcium	**3 %** (± 0.96)	3%	**3 %** (± 0.98)	4 %
Iron	**7 %** (± 2.17)	6%	**10 %** (± 3.10)	9%

It is interesting to note the impact that frozen processing has on the Vitamin C (Ascorbic Acid) content of the strawberry. Previous research suggested that there are changes in quality parameters such as anthocyanin content, total ascorbic acid, and total soluble sugars during cool storage (Cordenunsi, 2003). In this study the changes were more closely linked to the differences in the process of cooling storage and were agreed with those previous work. Another study also found that the loss of ascorbic acid occured during the first 15 days of storage (Sahari et al., 2004). Furthermore, prior study also found that the best storage temperatures for strawberries were at either -18°C or -24°C to preserve the qualitative characteristics of the berry (Sahari et al., 2004).

In the study conducted by Ayala-Zavala et al., (2004), higher antioxidant capacity, total phenolics, and anthocyanins were found in strawberries stored at 10° or 5°C in comparison to 0°C (Christina, 2005). This finding suggested that the antioxidant capability of strawberries was reduced after the strawberry underwent frozen processing. Another conclusion from the study was that shelf-life was longer at 0°C than either 5° or 10°C based on overall quality of the fruit. Since most frozen strawberries are processed at 0°C, it appears the industry has chosen to forgo antioxidant capacity in exchange for longer shelf life (Christina, 2005).

Nutritional information for freeze-dried strawberries is a little convoluted. Shown in Table 4.5 are the nutritional facts currently studied for freeze-dried strawberries (analytically done by SIRIM Qas International Sdn Bhd) and the results had been compared with the previous study. The first column shows the nutritional facts for a freeze dried strawberry product intended to be reconstituted before eaten. The second column is the nutritional information from prior study by Christina (2005) and the third column is for the novelty item called "Astronaut Strawberries" intended to be eaten before reconstitution. When comparing the above nutritional information to the nutritional information for one cup of fresh strawberries it could be seen that the reconstituted freeze-dried strawberries maintain approximately the same amount of calories, fiber and sugar. However, there was a drastic reduction in the amount of vitamin C in the reconstituted product.

In a study done by Pirker et al., (2002) it was found that there was an inverse relationship between the free radical contents of freeze-dried fruit and the water content of the fresh fruit; the higher the water content in fresh fruit the lower the content of free radicals in the freeze dried fruit. Furthermore, Piker et al., (2002) concluded that the free radical levels in freeze-dried fruits were approximately ten times higher than in frozen samples. This suggests that there must be free radical generation in the freeze-drying process. The generation of free radicals could create impact on the amount of Vitamin C (Ascorbic Acid) available in a freeze-dried product.

Table 4.5: Nutrients information (± standard deviation) for freeze dried strawberries

Nutrients	Freeze-Dried (Current Study)	Freeze Dried, ½ cup (9.5g) (Christina, 2005)	Freeze Dried 0.5 oz (Christina, 2005)
Calories	**45** (± 6.49)	36	50
Dietary Fiber	**2.5 g** (± 0.43)	1.4 g	n/a
Sugar	**5.2 g** (± 2.45)	4.7 g	n/a
Vitamin C (Ascorbic Acid)	**53%** (± 16.35)	47%	100%

Other than that, ascorbic acid concentrations were measured in strawberies and jackfruits that had undergone ultrasonic treatment before being proceeded into freeze-dried techniques. The results had been illustrated in Tables 4.6 and 4.7 and the comparison had been made between each treated samples.

Table 4.6: Ascorbic acid concentrations (± standard deviation) of strawberry samples

Samples Preparation	Ascorbic acid Concentration (Miligrams/mL) (Triplicates Analysis)	Ascorbic acid Concentration (Miligrams/100g of fresh weight) (Triplicates Analysis) (Danny et al., 2003)
Fresh frozen strawberries	8.66 (± 4.08)	27.1
Freeze-dried strawberries (-20^{o}C)	3.08 (± 1.45)	9.8
Freeze-dried strawberries(-20^{o}C/ Ultrasonic treatment)	3.65 (± 1.72)	Not applicable
Air-dried strawberries (60^{o}C)	Not detected	3.6

Concentrations of ascorbic acid (AA) were measures by HPLC because AA produces an oxidative-reduction reaction that contributes to the absorbance measurement in the Follin Ciocalteu assay (Danny et al., 2003). Levels of AA in fresh samples were consistently higher than the level of freeze-dried samples. A statiscally significant decrease in AA content was obesrved in freeze-dried and air-dried samples when compared to fresh frozen samples. AA content was not detectable in air-dried samples. Measurements of AA content in all sample preparations were different from other published values but still demonstrated consistent patern in AA decreasing content in freeze-dried fruits (Danny et al., 2003). As compared to previous study, current study just showed a slightly decreased AA content in freeze-dried strawberry sample. For freeze-dried strawberries samples that had undergone ultrasonic treatment after being frozen at -20^{o}C, the AA content was much higher than the samples that had not been treated by ultrasonic process. The ultrasonic treatment had helped to remove all the dirt and small particles from strawberry skin and this had caused to lower the decreasing of AA content in its samples. The study on quantitative nutritional analysis could not be compared with the previous study as the approach process and characteristics of the samples were different from each other.

Table 4.7: Ascorbic acid concentrations (± standard deviation) of jackfruit samples

Sample Preparation	Ascorbic acid Concentration (Miligrams/mL) (Triplicates Analysis)
Fresh Jackfurits	0.013 **(± 0.006)**
Freeze-dried jackfruits (-20^{o}C)	0.005 **(± 0.002)**
Freeze-dried jackfruits (-20^{o}C/ Ultrasonic treatment)	0.009 **(± 0.004)**
Air-dried jackfruits (60^{o}C)	Not detected

Besides that, freeze-dried jackfruit AA content had also being investigated in this study. The results indicated that, the techniques on preparing analysis of AA concentration for strawberry analysis can not be applicable to jackfruit, as it affected the analysis on those samples. The concentration of AA extracted from fresh jackfruit was lower than the amount extracted in fresh strawberries. It was shown that, fresh strawberry contains higher AA content than fresh jackfruit, thus making the strawberry as one of the most perishable fruit to the consumer. But for freeze-dried jackfruits which was treated by ultrasonic showed the same pattern of value when compared to the strawberries. Both samples proved that ultrasonic treatment could manage to preserve AA content in fruits. The AA content was much higher in those freeze-dried samples treated by ultrasonic than the normal freeze-dried samples.

Ultrasound assisted extraction (UAE) process enhancement for food and allied industries were reported previously (Kamaljit et al., 2008). This included herbal, oil, protein and bioactives from plant and animal materials (polyphenolics, anthocyanins, aromatic compounds, polysaccharides and functional compounds) with increased yield of extracted components, increased rate of extraction, reduced extraction time and with higher processing throughput. Ultrasound could enhance existing extraction processes and enable new commercial extraction opportunities and processes. New UAE processing approaches had been proposed, including, the potential for modification of plant cell material to provide improved bioavailability of micro-nutrients while retaining the natural-like quality, simultaneous extraction and encapsulation, quenching of the radical sonochemistry especially in aqueous systems to avoid degradation of bioactives

and potential use of the radical sonochemistry to achieve targeted hydroxylation of polyphenolics and carotenoids to increase bioactivity (Kamaljit et al., 2008).

Comparative measurement for air-dried jackfruit and strawberries were not available. AA was not detectable in both air-dried samples and it was due to the heating process that had degraded the AA contents in those fruits. The results for jackfruits samples could not be camparable to other previous study as different technique was applied in that process and nutritional information for freeze-dried jackfruits was a little convoluted. Vitamin C is a heat-sensitive bioactive compound in the presence of oxygen and gets degraded by oxidative processes,which is stimulated in the presence of light, oxygen, and enzymes like ascorbate oxidase and peroxidase (Davey et al., 2000).

The impact of freeze-drying and air-drying on total phenolics (TP) concentrations was also evaluated and compared to the TP content in fresh samples that were frozen and stored at -20^{o}C. In a previous study of peaches, it was demonstrated that freezing and storing at -12^{o}C for up to 6 months produced no significant decrease in TPs as compared to fresh samples (Danny et al., 2003). Therefore, levels of TPs in air-dried and freeze-dried samples of strawberries and jackfruits were compared to those found in frozen samples because the fresh samples were not available for long shelf life time storage to be analysed.

The average TP content of frozen, freeze-dried and air-dried strawberries were 270.5, 231, and 28.7 mg/100 g of fresh weight, respectively (Figure 4.24). On a dry weight basis, average levels of TPs reported here for dried strawberries were not consistent with previously reported values of 1600-2410 mg/100 g of dry weight (Danny et al., 2003; Kahkonen et al., 2001). In contrast, the level of TPs was reported for frozen strawberries which was 270.5 mg/100g of fresh weight was 11%, consistent with previously reported value of 241 mg/100g of fresh weight (Danny et al., 2003).

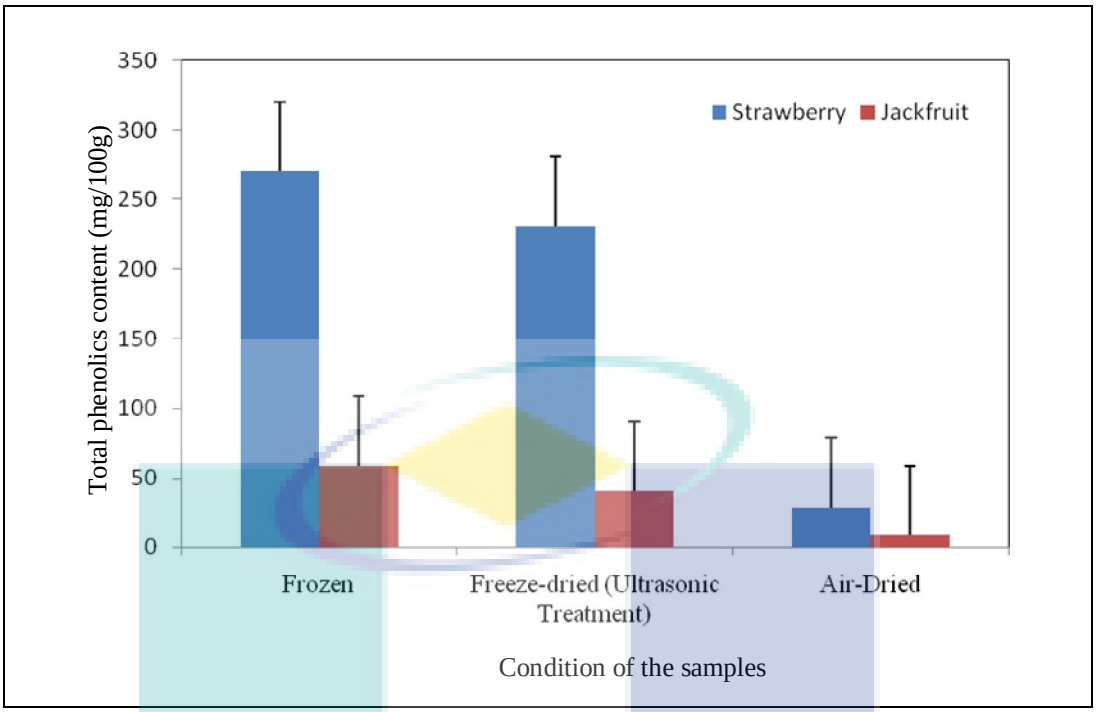

Figure 4.23: Total phenolics (mg/100g of fresh weight) in frozen, freeze-dried and air-dried strawberry and jackfruit

The levels of TPs in frozen, freeze-dried, and air-dried jackfruit methods were 58.5, 40.7, and 8.4 mg/100g of fresh weight, lower than levels measured in strawberies samples, respectively (Figure 4.23). On average, the TP content of air-dried jackfruit was 86% lower than that of frozen jackfruits. There was no significant difference between frozen and freeze-dried jackfruits samples from previous study that could be used for comparison.

The highest levels of TPs were consistently found in the extractions of frozen samples, followed by freeze-dried and then air-dried. In general, air-drying at temperatures more than 60°C is regarded as unfavorable due to the possibility of inducing oxidative condensation or decomposition of thermolabile compounds, such as (+)-catechin (Danny et al., 2003). Conversely, freeze-drying may lead to higher extraction efficiency of TPs because freeze-drying can lead to the development of ice crystals within the plant matrix. Ice crystals could result in a greater rupturing of plant cell structure, which may allow for better solvent access and extraction (Danny et al., 2003; Keinanen et al., 1996). With air-drying there was little or no cell ruptures and

there was the added effect of heat, which could cause losses in phenolics and ascorbic acid (Danny et al., 2003).

Various observations on TPs contents had been reported earlier by Gonzalez-Aguilar et al., (2007) in mango ("Haden" variety), as did Hagen et al., (2007) for apple. According to Frohnmeyer et al., (2003) and Gitz et al., (2004), UV irradiation induced the accumulation of phenolic compounds in plants as a defense mechanism against irradiation. However, the increase in TP could also be attributed to the phenylalanine ammonialyase activity, which is one of the key enzymes in the synthesis of phenolic compounds inplant tissues. Stevens et al., (1998) and Brown et al., (2001) found an increased in the activity of phenylalanine ammonialyase in peaches and cabbage seeds after UV exposure. The activation of this enzyme in mango fruits had been strongly correlated with the increased of phenolic content of the fruits (Gonzalez-Aguilar et al., 2007). Increased in phenolic compounds on irradiation of plant produce had also been attributed to depolymerization and dissolution of cell wall polysaccharides, which facilitated higher extractability (Bhat et al., 2007). This might also hold true in this present observations, wherein UV analysis might have facilitated higher extractability. However, it should be noted that antioxidants were not only phenolic based, but other compounds might also contributed towards the antioxidant activity (phytic acid, selenium, tocopherol) which needs to be further explored. UV analysis could be useful for enhancing the nutritional value of fresh-cut portions of these two fruits and play a positive role in preventing several physiological and pathological processes in foods.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Ice crystals in frozen strawberries and jackfruits were quantitatively investigated using x-ray micro-computed tomography. The x-ray technique is able to visualize the effect of freezing on strawberry and jackfruit after they were freeze dried to create voids in that foods neither on the direction in which that strawberries and jackfruits were frozen (normal and rapid freezing) nor the freezing rate of the strawberries and jackfruits (increasing distances from the cooling surface and thickness of the samples).

Ice crystals in thin samples had the smallest ice crystal size and the samples that isolated from the cooling surface had the slowest freezing rate thus creating the biggest ice crystals in frozen strawberry. Larger ice crystals and broader crystals size distributions were seen at the top of the sample than the centre; while at the bottom of the strawberry samples, the ice crystals formed were much smaller. The result suggested that the link of freezing rate that affect microstructures of these frozen strawberries. The interrelationship between ice crystal and strawberry microstructure in each treatment and freeze dried technique is the best way for measurement of the voids left by ice crystals.

The x-ray microcomputed tomography (XMT) techniques employed offered the opportunity of studying the whole product without any preparative manipulation which could mask the freezing effects. The results obtained should be useful to understand how and why freezing causes damage to fruit tissues and, therefore, to design optimized

freezing treatments which would minimize this damage. The data shown in the results and discussions had demonstrated the scope of XMT in observing ice crystals formation in strawberry and visualization image of jackfruits microstructure as the validation results to proved XMT ability to distinguish the differences between materials and void surface based on density differences. Throughout this research not only imaging analysis had been investigated, nutrient characteristics of freeze-dried products using the freeze-drying technique also had been analyzed in order to optimize this drying process which could minimize the damage of the fruits properties.

The particular characteristics of strawberry (air content and distribution in the tissues, pH, sugars profile, ascorbic acid and total peholics content) had determined the freezing techniques effects described in this reseacrh. XMT techniques had been found to be very useful to better understand the changes that take place during freezing , freeze-dried and air-dried processing. Those results demonstrated a statistically relevant trend of total phenols (TPs) level content and ascorbic acid (AA) analysis in strawberry and jackfruits samples. More interestingly, our results indicated that TPs and AA were still preservable in those samples and quite closed to the fresh frozen samples. The TPs value in freeze-dried strawberry was 82% higher than the jackfruit samples which was 231 mg/100g fresh weight. Besides that, the TPs value was much higher in freeze-dried samples as compared to the air-dried samples for both fruits. Freeze-drying may lead to higher extraction efficiency of TPs because freeze-drying could lead to the development of ice crystals within the plant matrix.

AA analysis had shown that there was just a slight decrease of that content in freeze-dried strawberry samples than in the fresh frozen samples as compared to previous study. Fresh frozen samples consists 149% AA content and the freeze-dried strawberry samples could still preserve 53% of that AA content. There was a slight decrease, however, as it was found that freeze-dried techniques still could preserve higher AA content as compared to air-dried technique in which the AA content was not at all detectable in those samples.

Ultrasonic treatment to those sample before freeze-dried process had been implemented throughout this study. The results indicated that higher AA and TPs content were preserved in those samples that were treated using ultrasonic. Ultrasonic could enhance existing extraction processes and enabled new commercial extraction opportunities and processes. New ultrsonic treatment approaches had been proposed, including, the potential for modification of plant cell material to provide improved bioavailability of micro-nutrients while retaining the natural-like quality, simultaneous extraction and encapsulation, quenching of the radical sonochemistry especially in aqueous systems to avoid degradation of bioactives and potential use of the radical sonochemistry to achieve targeted hydroxylation of polyphenolics and carotenoids to increase bioactivity.

There are many benefits to freeze-drying. First of all, the product retains its initial properties such as shape, appearance, taste, color, flavor, texture, and biological activity. Secondly, freeze-dried products have a high rehydration capacity. These factors paired with the high yield and a longer shelf life make a freeze dried product superior to the fresh one. Furthermore, there is a reduced weight for storage, shipping and handling. All in all, freeze drying makes a highly perishable product extremely storable and easily to transport anywhere in the world and XMT techniques is one of the novelties for this research as it would help in design better freeze-dried technique and is very useful to better understand the changes that take place during processing by using the image analysis on mirostuctural changes in those samples.

5.2 RECOMMENDATIONS

Results obtained from these observations had shown that the link of freezing rate that affect on microstructures of these frozen strawberries. The interrelationship between ice crystal and strawberry microstructure in each case and freeze dried technique is the best way for measurement of the voids left by ice crystals. Since current research is limited by facilities constraint, future research can also be extended to study on temperature profiles for frozen strawberry samples at freezing temperature for different thickness of the samples in each freezing technique from its cooling surface. The overall trends of the temperature transients of different thickness affect on different

morphology of ice crystals can be respectively clarified. Other than that, using the data provided in this research, further design, and analysis and processing of frozen strawberry can be done and it gives the possibility to examine the internal structure of these samples in 3-D. The freeze drying techniques on frozen strawberry before scanning can be improved in order to find the optimal condition for freeze drying the strawberry.

There is also potential in extending the study on X-ray micro computed tomography (XMT) as one of the promising techniques that can help in distinguishing the low density differences of solids and liquids inside any material rather than any other visualization techniques. This possibility helps in recognizing these two different voids in the materials. The preliminary observation on this XMT provides a new technique in order to study on different voids occuring in processing materials. Further investigation on reducing or optimizing the process can be analyzed during scanning process and its ability to clarify the exact volume of liquid or other particles in all types of materials.

Although freeze-drying seems like a great process, there are some drawbacks to the process. First of all, reconstitution can be an issue depending on the use of the freeze dried fruit. Research has found that the functional properties of the fruit can be altered as a result of different concentrations of reconstitution media. This may be beneficial for producing formulated foods with a high added value. However, it may be detrimental when foods must be reconstituted for consumption. When using a reconstituted product for consumption, it is important to consider the time, process, type and concentration of the reconstitution media which all would have an effect on water uptake.

Currently there are limited uses for freeze dried strawberries. Most of the freeze-dried processed fruit is used in cereals. Berry Burst Cheerios, Honey Bunches of Oats with Strawberries and Special K with red berries are some of the examples. Freeze-dried strawberries are also sold as a novelty item labeled as “Astronaut Food” and as a dessert for backpackers or others planning to be out in the wilderness for lengthy periods. With the change in the food guide pyramid, there is a potential increase for this type of food

products. Snack bars which now use fruit flavored pieces might include freeze-dried fruits as the demand for more fruits in the diet increases.

Strawberries have proven to be a rich source of ellagic acid. Furthermore, there has been a lot of research on the benefits of ellagic acid. Unfortunately there does not seem to be any research on the consumption of strawberries as a source of ellagic acid for health whether it can be a good source for obtaining health benefits of ellagic acid. Other than that, there is still a lack of research on our local seasonal fruits. This should be taken into consideration by all of our local food researchers to improve and commercialise our local products to the world by using our own technologies. In the current literature there seems to be limited information about nutritional changes that occur during processing especially for our local fruits such as jackfruits.

Although strawberries are available all year round, the majority of individuals consume more processed strawberries over fresh strawberries for many months in the year. Current research suggests that freezing temperatures and length of time frozen have different impacts on the antioxidant levels of the fruit. More research should be conducted to examine the best combination of the two in order to maintain nutritional integrity. In addition, researchers should look at the changes in ellagic acid content of strawberries during processing and also nutritional analyses on jackfruit as one of the most promising local seasonal fruit that can be commercialised.

There seems to be very little information available about the freeze-drying processing technique. One study concluded that there was free radical generation during this type of processing. If this was true, more studies on that process and free radical generation should be done in order to know whether it caused a reduction in Vitamin C or not. Furthermore, it is necessary to find out about the occurence of nutritional changes after undergoing this process. Lastly, to increase the demand for freeze-dried products, the industry should focus on making it economically feasible to replace fruit flavored pieces with real strawberry pieces in snack bars.

As a result of this research there are many factors that consumers should be aware of when consuming strawberries. The consumers should be aware of the health benefits that can be received by including strawberries as a part of their diet. The best source for these health benefits come from fresh strawberries. However, when fresh ones are not available, it is appropriate to supplement the diet with frozen or freeze-dried strawberries.

Consumers should be aware that nutritional changes do occur during processing. Both frozen strawberries and freeze-dried strawberries lose vitamin C, with the latter losing the most. Currently, it is unknown of the impact of these processes was on the antioxidant capacity. However, it appears that both processes lead to a reduction in the antioxidant capacity. At this point, there is not enough information to conclude which process leads to a greater destruction of antioxidants.

Finally suggestion for consumers is to be aware when purchasing frozen berries. Diabetics and those who must monitor their sugar or carbohydrate intakes should be aware of the added sugar in frozen strawberries. If frozen strawberries cannot be purchased unsweetened, it is best for these individuals to buy fresh strawberries in season and then freeze them at home to regulate the amount of sugar added.

All in all, rheological measurement and other physical techniques should be improved in the same experiments and more specifically and detailed design of experimental works will offer the possibility of obtaining better detailed of structural information of voids in food materials.

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APPENDIX A

MICROSTRUCTURE ANALYSIS USING CTAN AND IMAGE J SOFTWARE

CTAN ANALYSIS STEPS

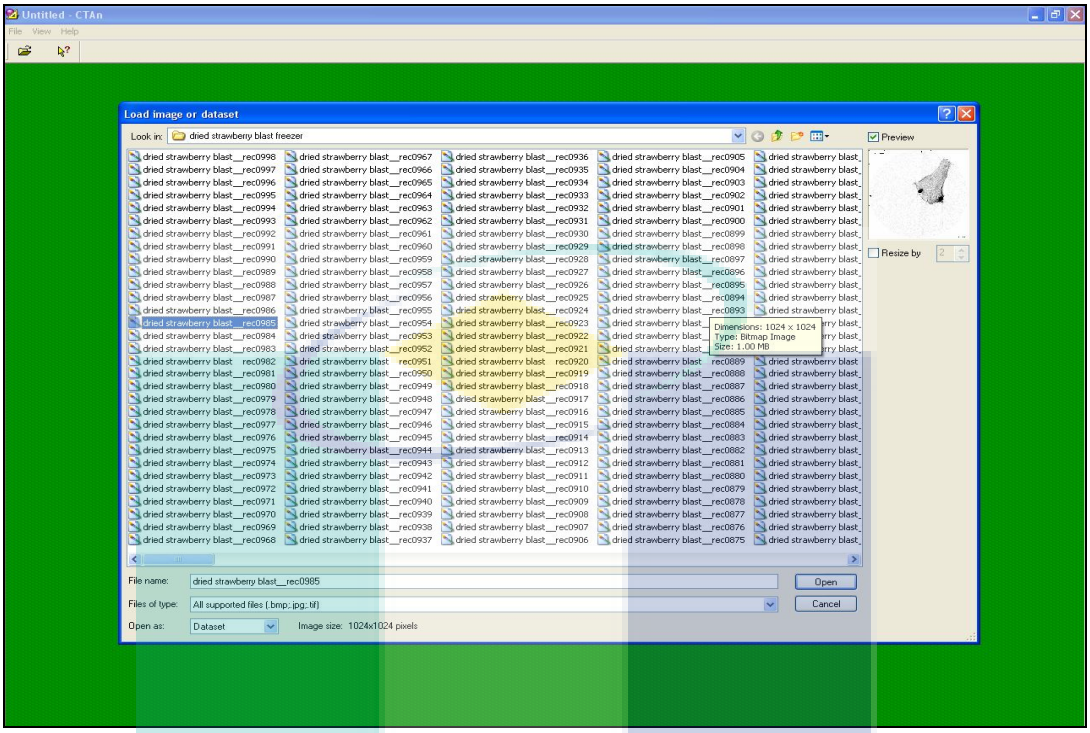


Figure A.1: Loading the reconstruction raw image

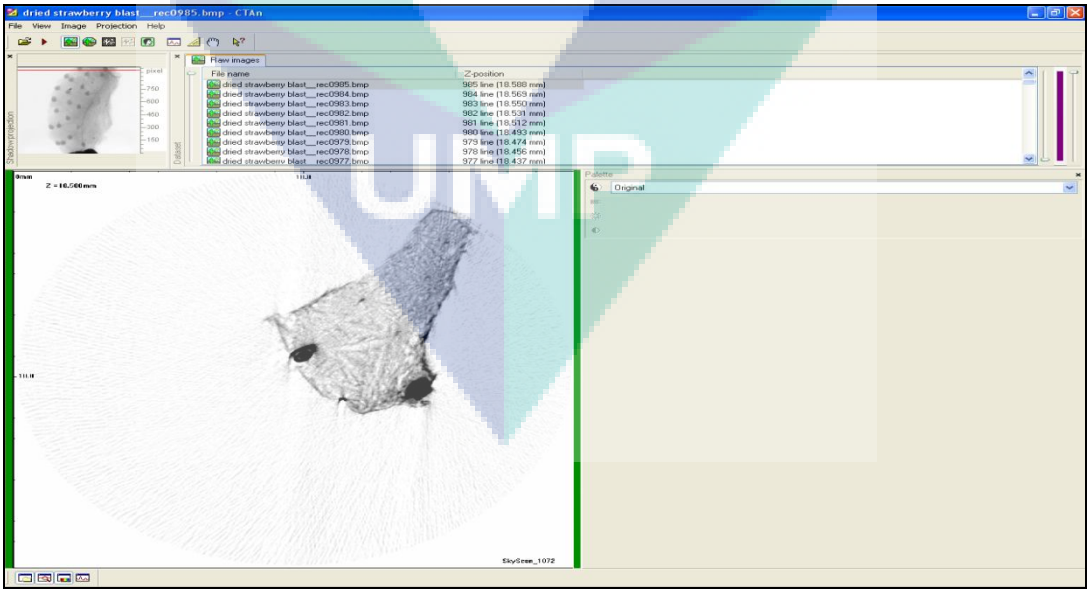


Figure A.2: Select the region of interest

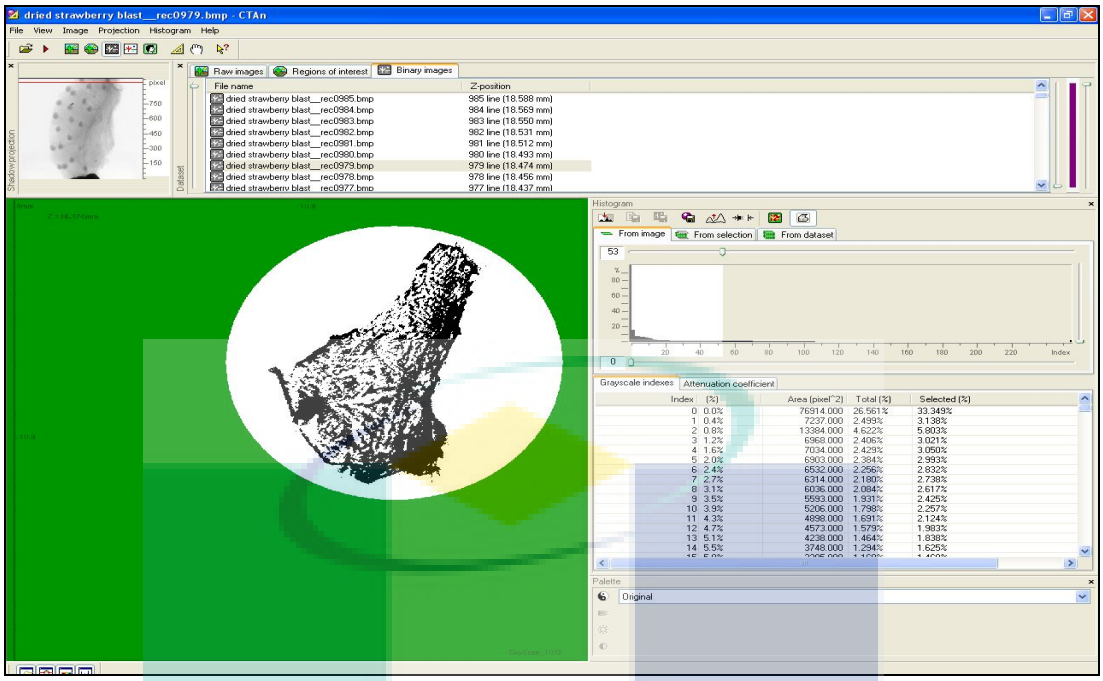


Figure A.3: Region of interest analysis

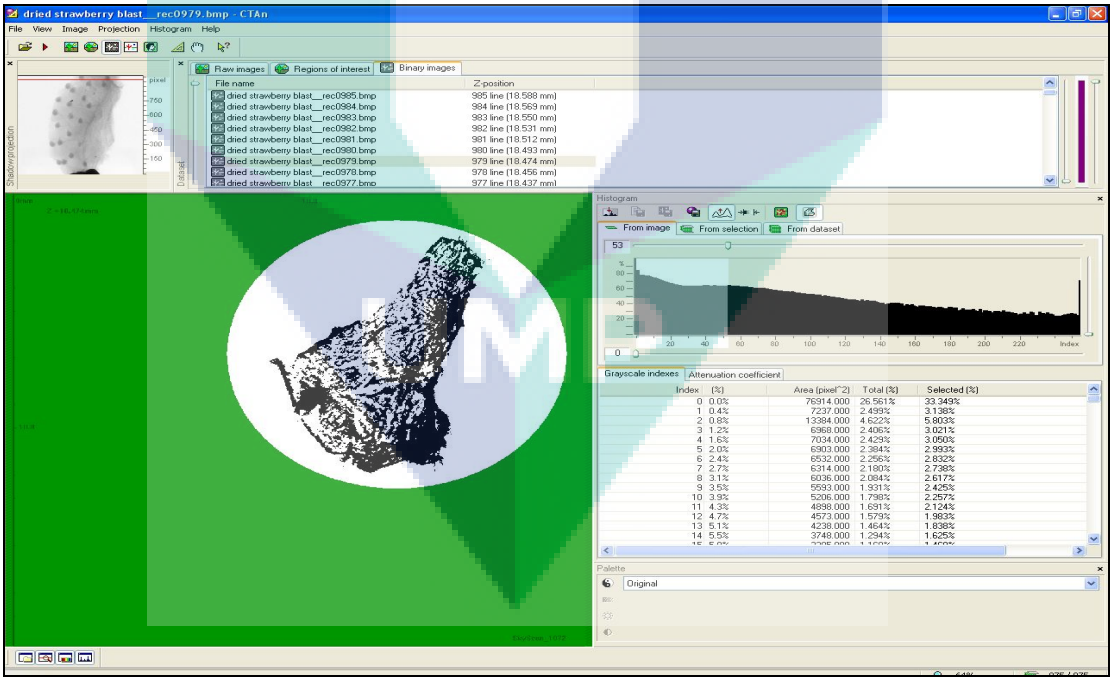


Figure A.4: Threshold of the image

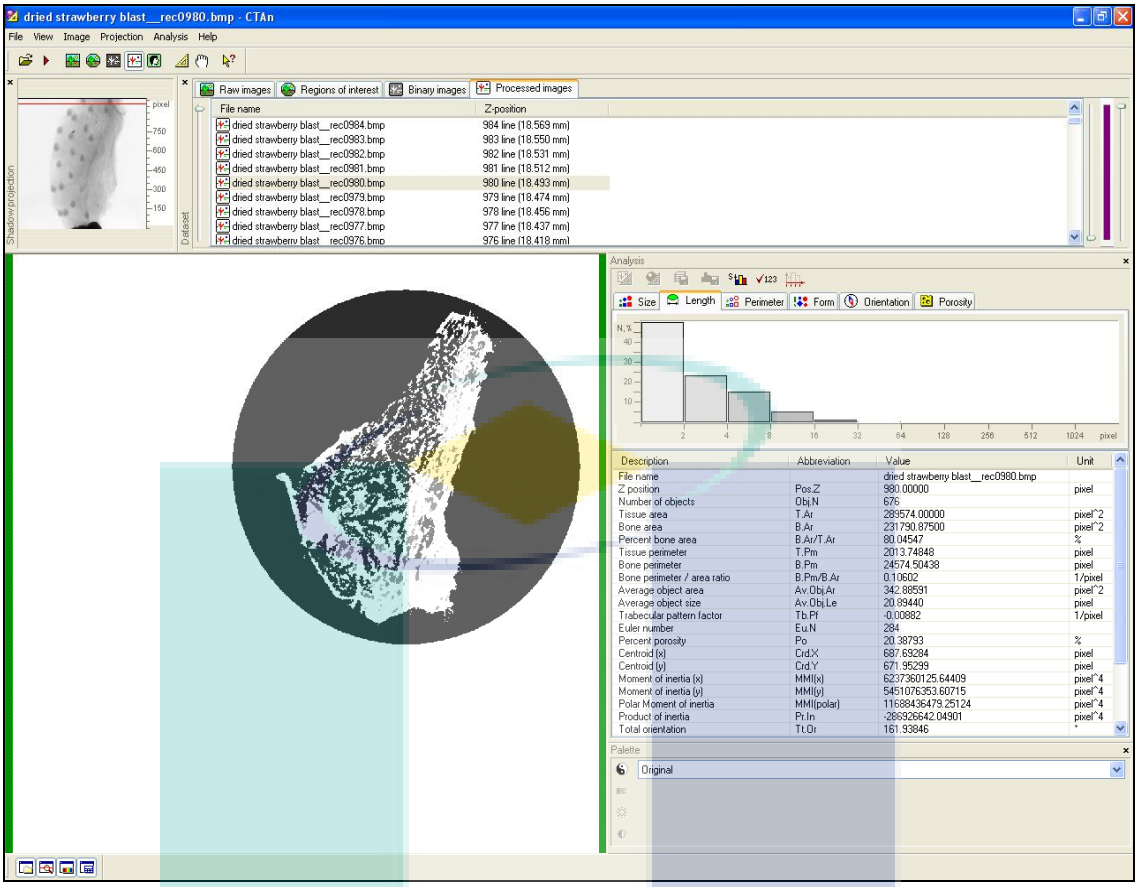


Figure A.5: Custom processing of the image analysis

CTAN / CTVOL Software Package: Getting Started

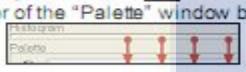
SkyScan, August 2003, Aartselaar, Belgium.

Skyscan's new software package, for image analysis and visualization of the results from micro-CT system scans, contains two programs: **CTan** for 2D visualization and 2D/3D analysis, and **CTvol** for realistic 3D visualization. If the software is supplied as a zip-file, unpack it into any existing or new folder of the local hard-drive. Start CTAn first. To use the two programs together, for direct export of the selected object volume-of-interest (VOI) to 3D-visualization, connect CTvol to CTan: in CTan select "File"->"Preferences"->"Tools" and define CTvol.exe as the 3D visualization program.

Open any set of micro-CT results by the button in the toolbar. The shadow image (if present) and list of files will be shown in the top part of the program window and one of the cross sections in the bottom part. You can animate through the selected dataset levels by pushing or using the slider on the left side of the files list. Right mouse click in the cross section image to open a submenu for image zoom. Drag and drop the left mouse button in the cross section image or the projection image to measure distances. Different color look-up tables can be selected in the "Palette". Click the sign to invert the image.

After loading a dataset several buttons will appear in the main toolbar: : original image view, : selection of region or volume of interest, : conversion of images to binary for analysis, and (appearing after conversion to binary) : image analysis. If you wish to select a region/volume of interest, click the button and draw with left mouse button held any shape of the region directly on the cross sectional image. This shape can be copied to all cross sections in the dataset by the toolbar button. To select a 3D volume of interest you can draw different regions in different cross sections, and software will interpolate them automatically through all intermediate cross sections. Use the toolbar button with pop-down submenu to select regular shapes for the region-of-interest. Volume-of-interest can be limited in the vertical direction by two sliders to the right of the files list. Double-click on the blue bar between these sliders to enter upper and lower limits numerically. One can reset all regions-of-interest by button to "start again" with VOI selection.

Go to conversion to binary images with the button. Open the "Histogram" dialog window by dragging down the top border of the "Palette" window by the mouse:



Select the upper and lower global threshold levels by sliders above and under the histogram, or use the button in the local toolbar for automatic suggestion (finds first value containing voxels). The white part of the binary images represents solid objects for the subsequent 2D and 3D analysis. The grey level histogram shown uses the region / volume of interest if selected. This histogram can be saved by the button.

Use the button in the main toolbar for image analysis. Distributions of size, perimeter, form, orientation and internal porosity from the current 2D-image are shown as color-coded histograms in the "Analysis" part of the program window. Colors of the histogram's bars match the colors of the objects in the image with corresponding 2D parameter values. To enhance this color coding of the 2D histogram select "color" or "color2" from the "Palette" drop-menu. To see the results from another cross section, select it in the list of available files. Use the local toolbar or right mouse button in the "Analysis" area to start full individual 2D object analysis from the current image (button) and to start full 3D analysis (button) from the selected volume of

Figure A.6: Summarization of step involved in image analysis using CTAN software

interest. A further 2D analysis of all selected dataset images is possible by selecting the button, which will create a tabular comma-delineated text file.

Within the 3D analysis box select the required parameters. A data text report can be saved in either list or tabular (line-by-line) format (comma delineated), the latter suitable for opening by Excel. At the bottom of the box choose either "text file" (standard list report) or "single line of text file" (line-by-line tabular) as formats for auto-saving of analysis results. The tabular format text file allows saving of the results of several analyses adding each time a new line of text.

A report can be printed once 3D analysis is complete. To format the printed report go to "File"->"Print Layout" where you can choose which text and image components to include in the report.

To send the selected volume of interest to CTvol for realistic visualization, go back to the binary images by the button in the main toolbar (or the tab above the dataset window) and then push the button in the local toolbar. We recommend that you to select a small volume of interest in the central part of the object for your first visualization attempts, to avoid memory limitation and slow reaction of object movements in 3D-space. Select "Launch associated program to show model" in the "Create 3D Model" dialog for immediate viewing after model creation. CTan will export a 3D-model of the selected part of the object into the **CTvol** program for visualization. The CTvol program starts in a separate window with its own controls as explained below. It can also be started independently. CTvol can show 3D-models from the following files: CTM (from CTan), P3G (from ANT) and STL (from ANT and many other programs). The CTvol toolbar contains buttons to select object movement modes and object(s)/space properties: : object movement mode, : camera movement mode, : show/hide objects(s) properties dialog, : show/hide stage properties dialog, : show/hide light properties dialog. Object and camera can be moved by the mouse. By holding the right mouse button one can rotate the object(s) or camera around the object(s) depending on the selected mode in the toolbar. The mouse wheel always changes the distance from camera to object(s) – the directionality of this control can be selected in preferences. The left mouse button can shift object(s) in the case of selection for object movement , or zoom in / zoom out in the case of camera movement being selected. Double click on either mouse button to switch the program between object movement and camera movement modes.

The object properties dialog allows adjustment for the selected object of surface color and other visual properties, and the object can be set as movable/unmovable and visible/invisible. One predefined object – the plate – is generated automatically and can be used to cut the selected object by means of the "cut by plane" button.

The stage properties dialog allows adjustment of the camera viewing angle and selection of background: one specific color, 3D color box or an image box rendered by 6 images from the supplied or user defined files (with selected path+prefix+ "top", "bottom", "left"...). It allows also display of the object(s) in stereo mode for red-cyan or red-green glasses (use in the toolbar for quick ON/OFF stereo) and the adding of fog with adjustable density to the scene.

The light properties dialog allows adjustment of the color and direction of the light for object(s) illumination.

Making movies: The toolbar button opens the "Flight recorder" window. This option stores a number of scenes (keyframes) with smooth interpolation in between. The resulting movie can be shown directly on the screen and saved to file.

Angular resampling: The toolbar button allows creation of a new resampled set of cross sections from the original dataset at a different orientation (activated if connection to the original dataset is available). The new set of cross sections will follow the orientation of the cutting plate and will be located symmetrically around it. The number of cross sections and the space between them can be selected in the associated dialog.

The speed of image analysis in CTan and the speed and maximum object size for visualization of 3D-objects in CTvol are determined by the available resources in your computer.

Figure A.7: Continued summarization of step involved in image analysis using CTAN software

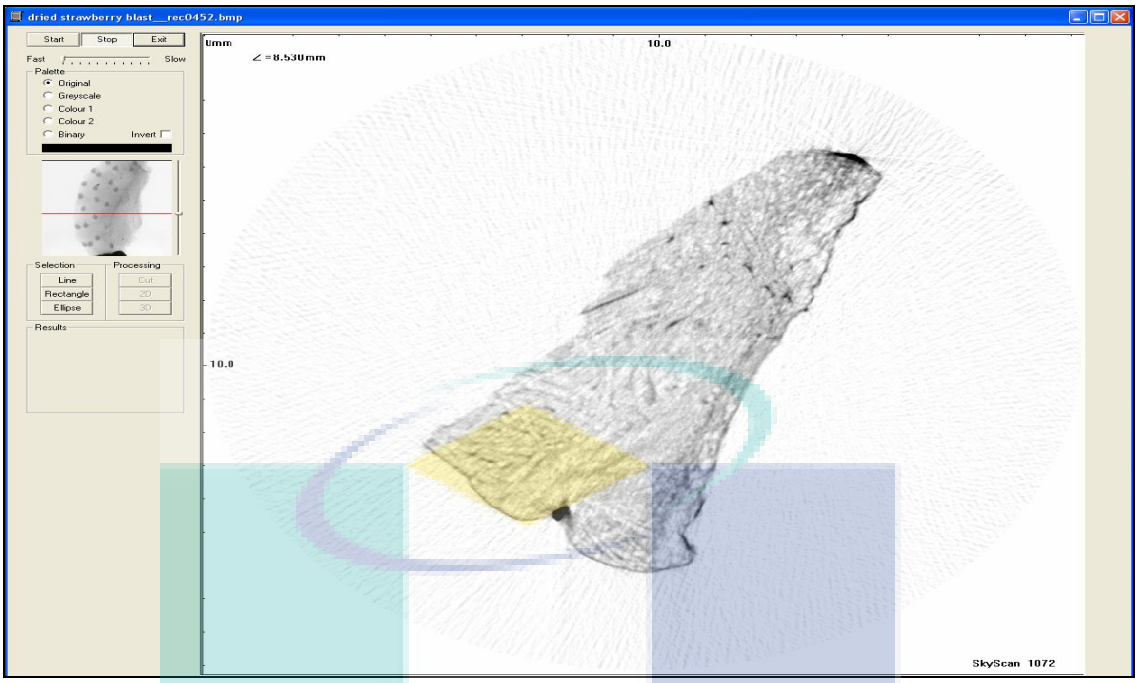


Figure A.8: Image viewer by TVview software.

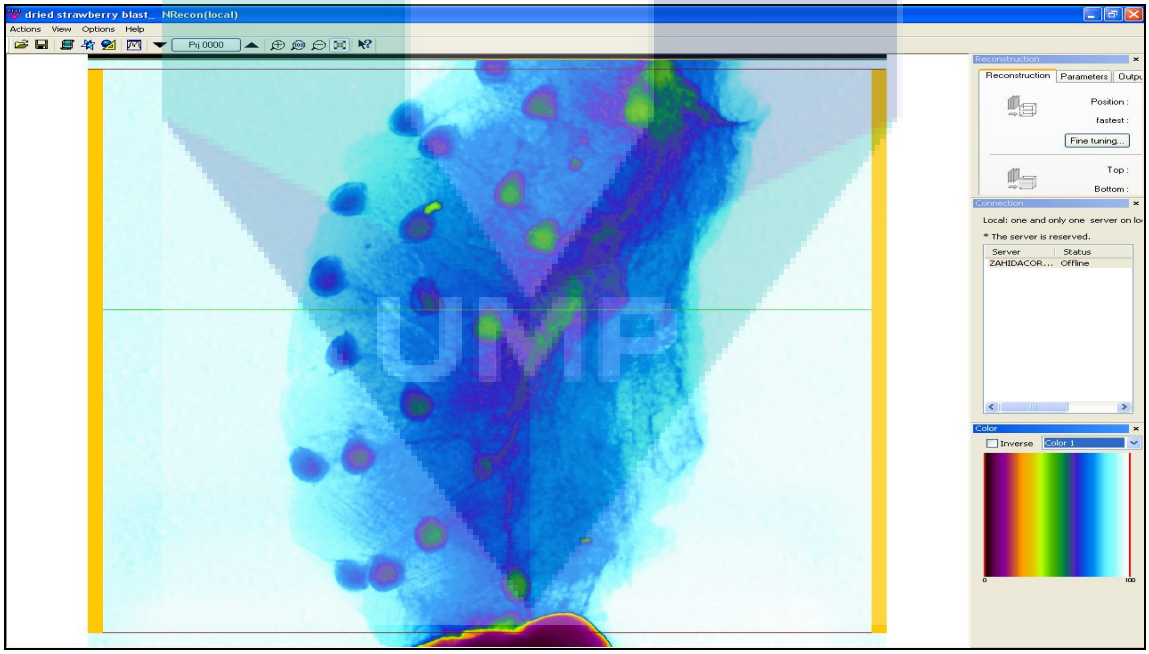


Figure A.9: Image reconstruction by using NRECON software

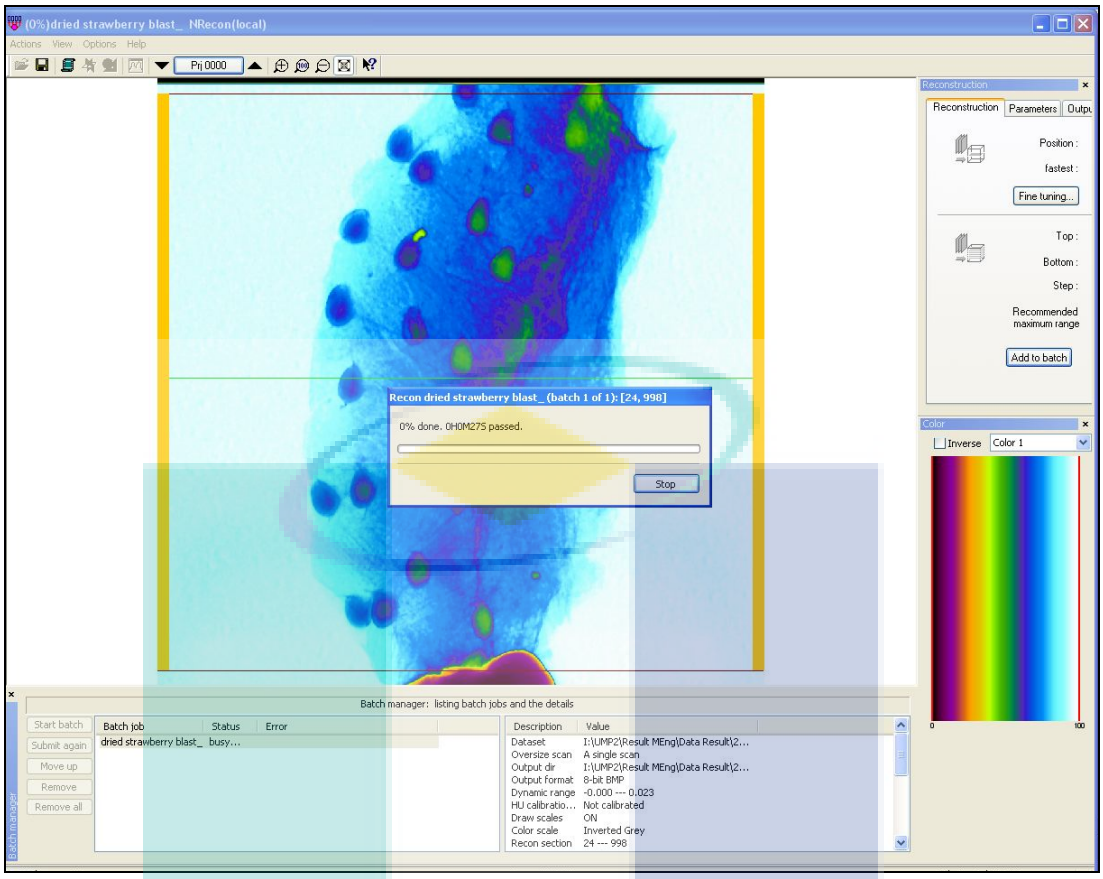


Figure A.10: Image reconstruction analysis

IMAGE J ANALYSIS STEPS

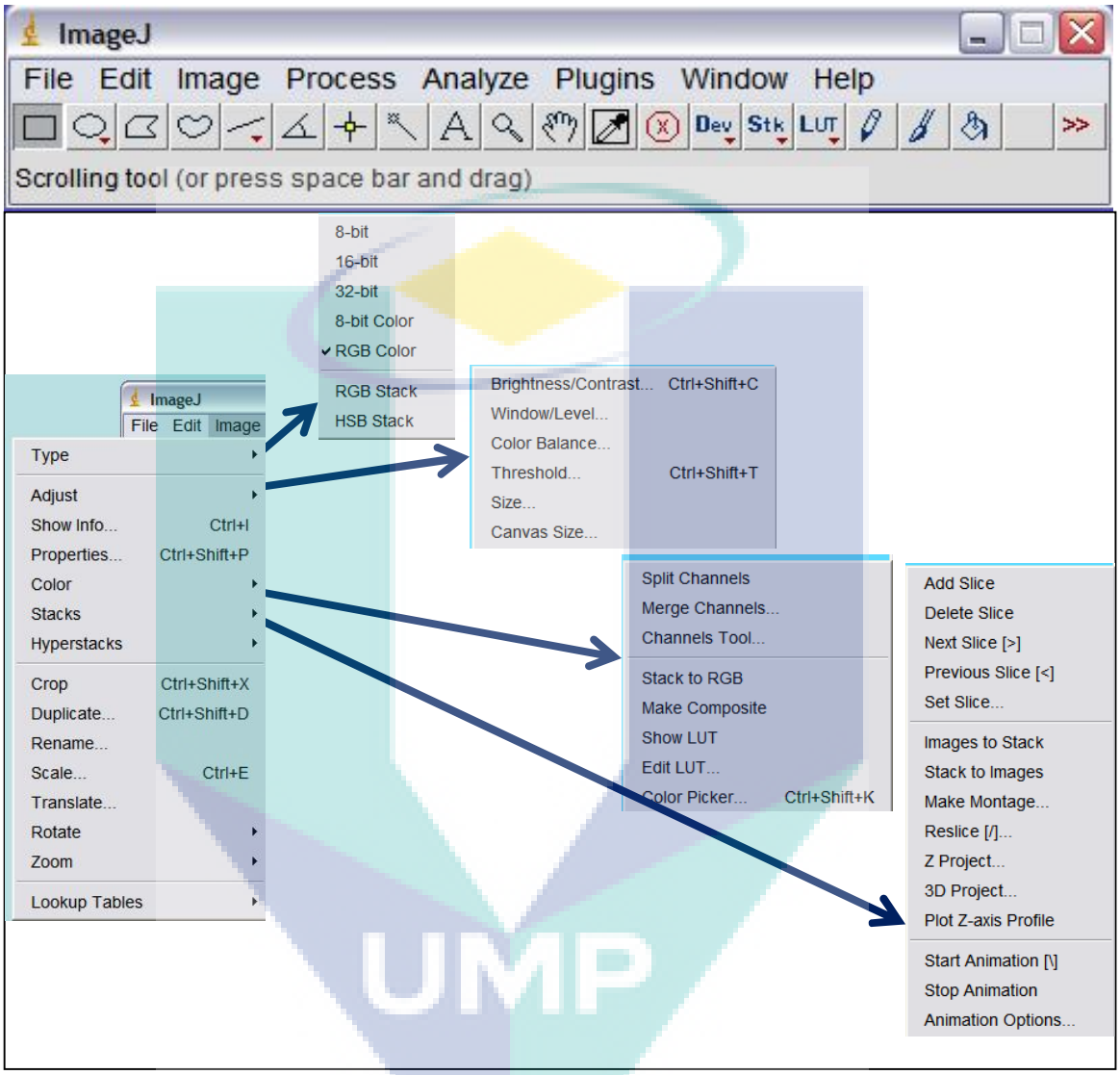


Figure A.11: Image J toolbars analysis

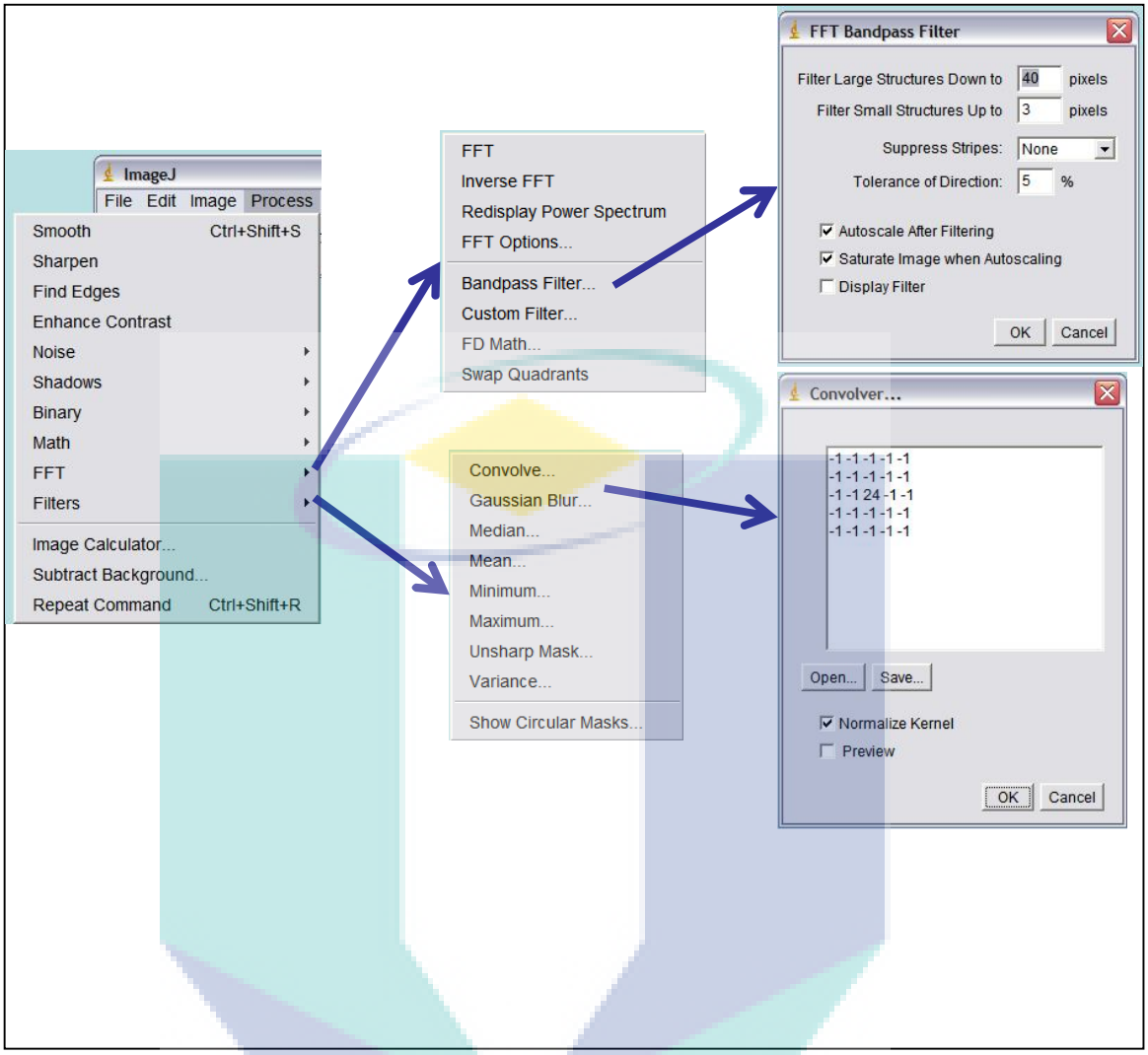


Figure A.12: Image J processing analysis

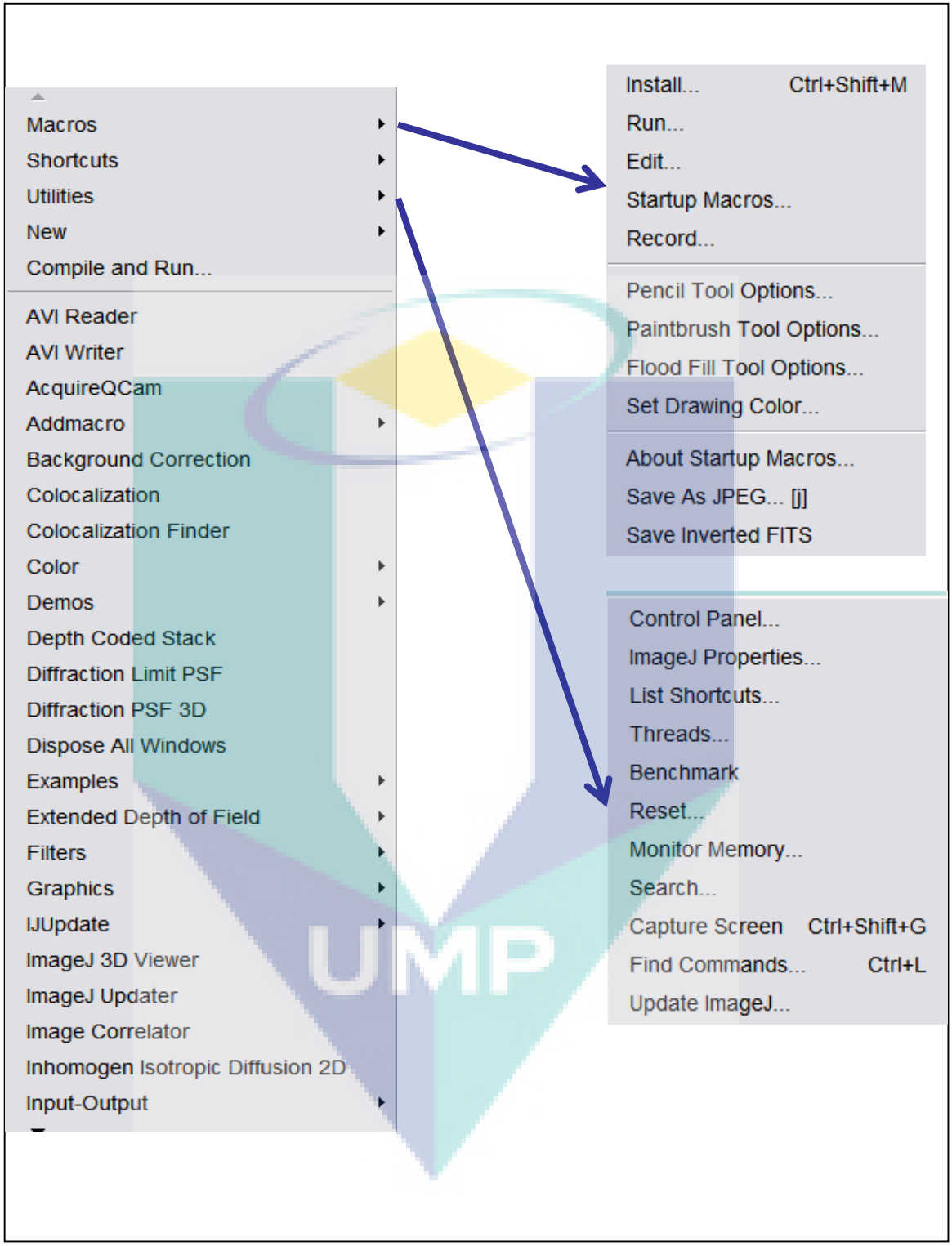


Figure A.13: Image J applications

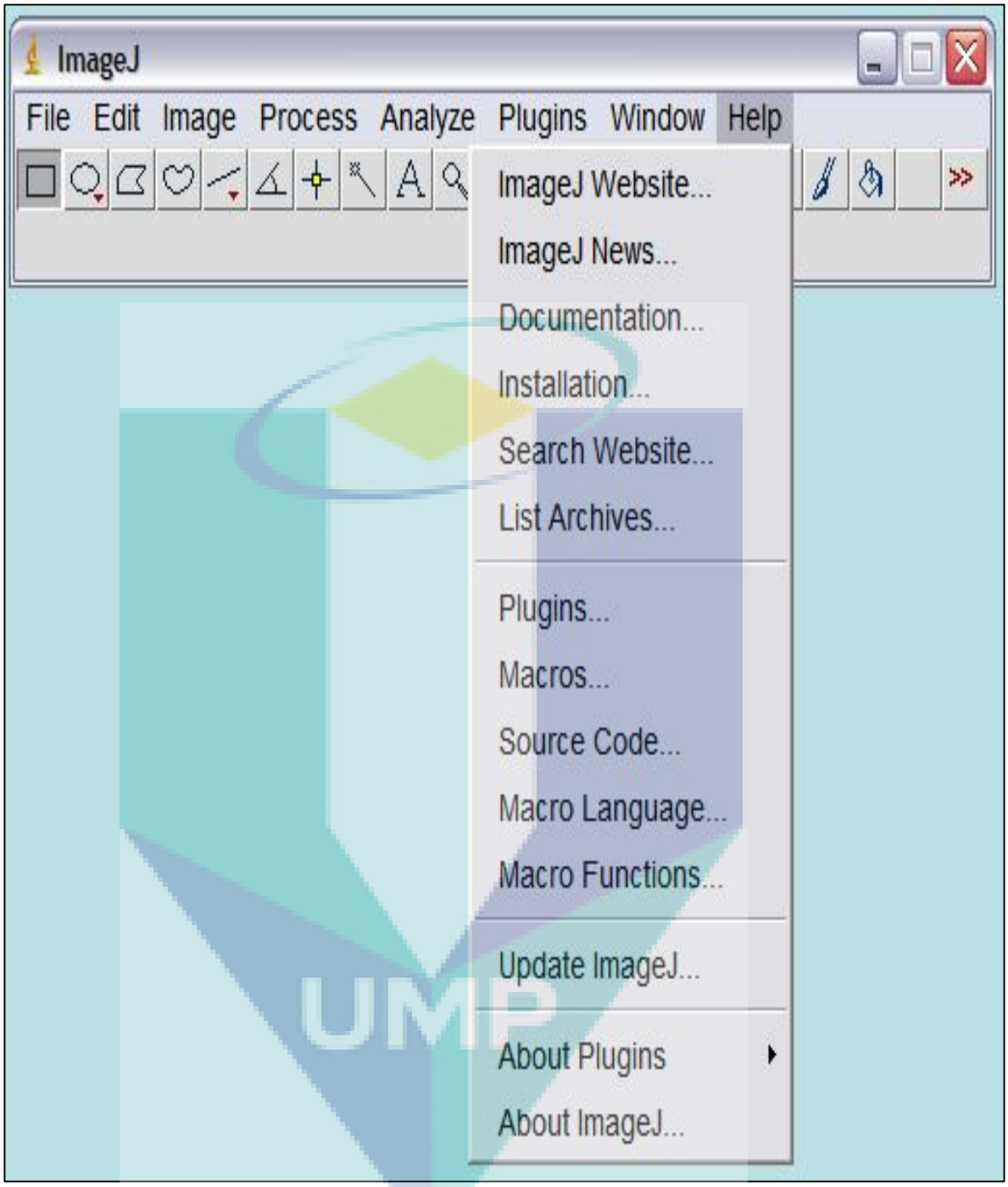


Figure A.14: Image J plugins analysis

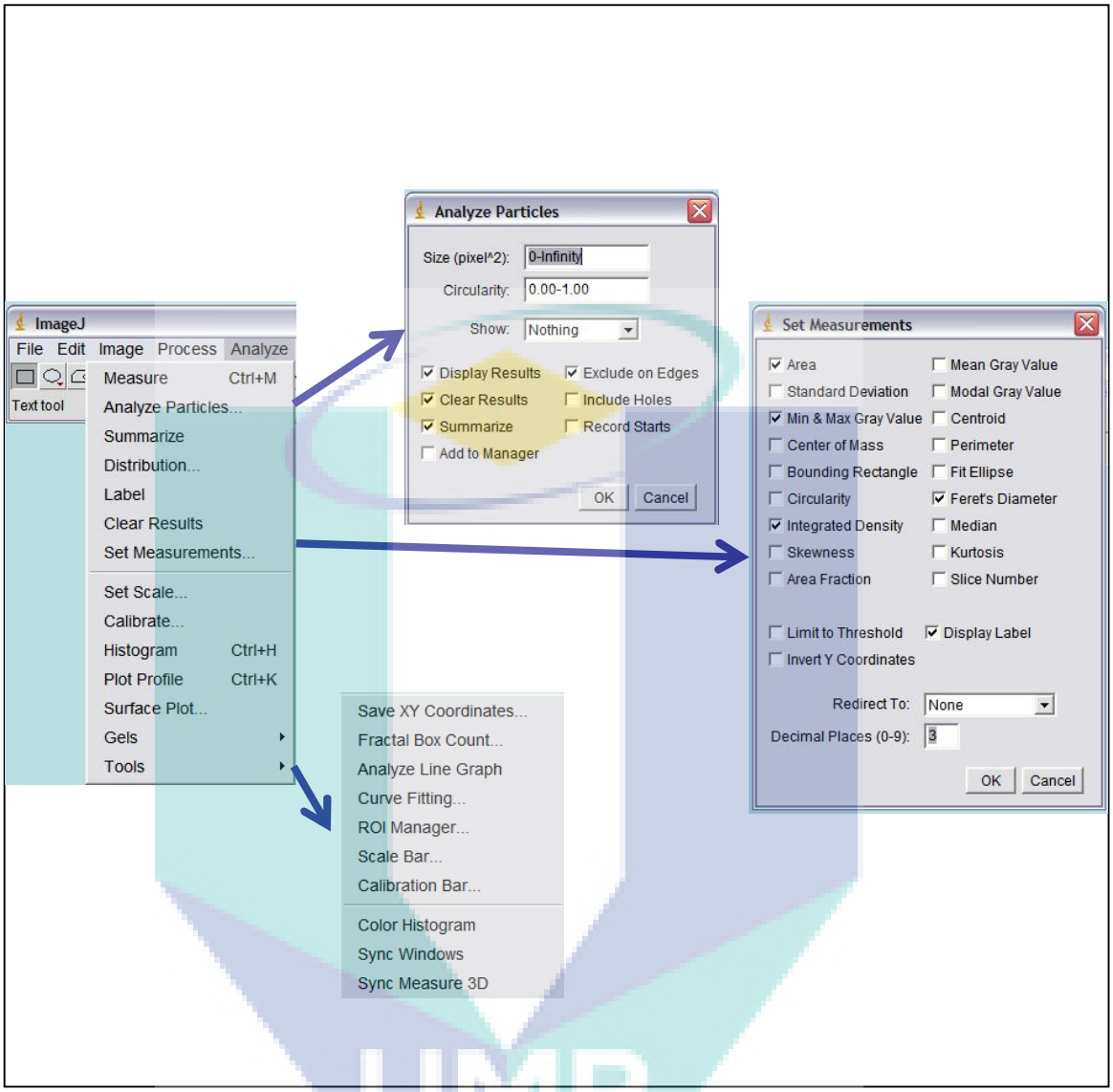
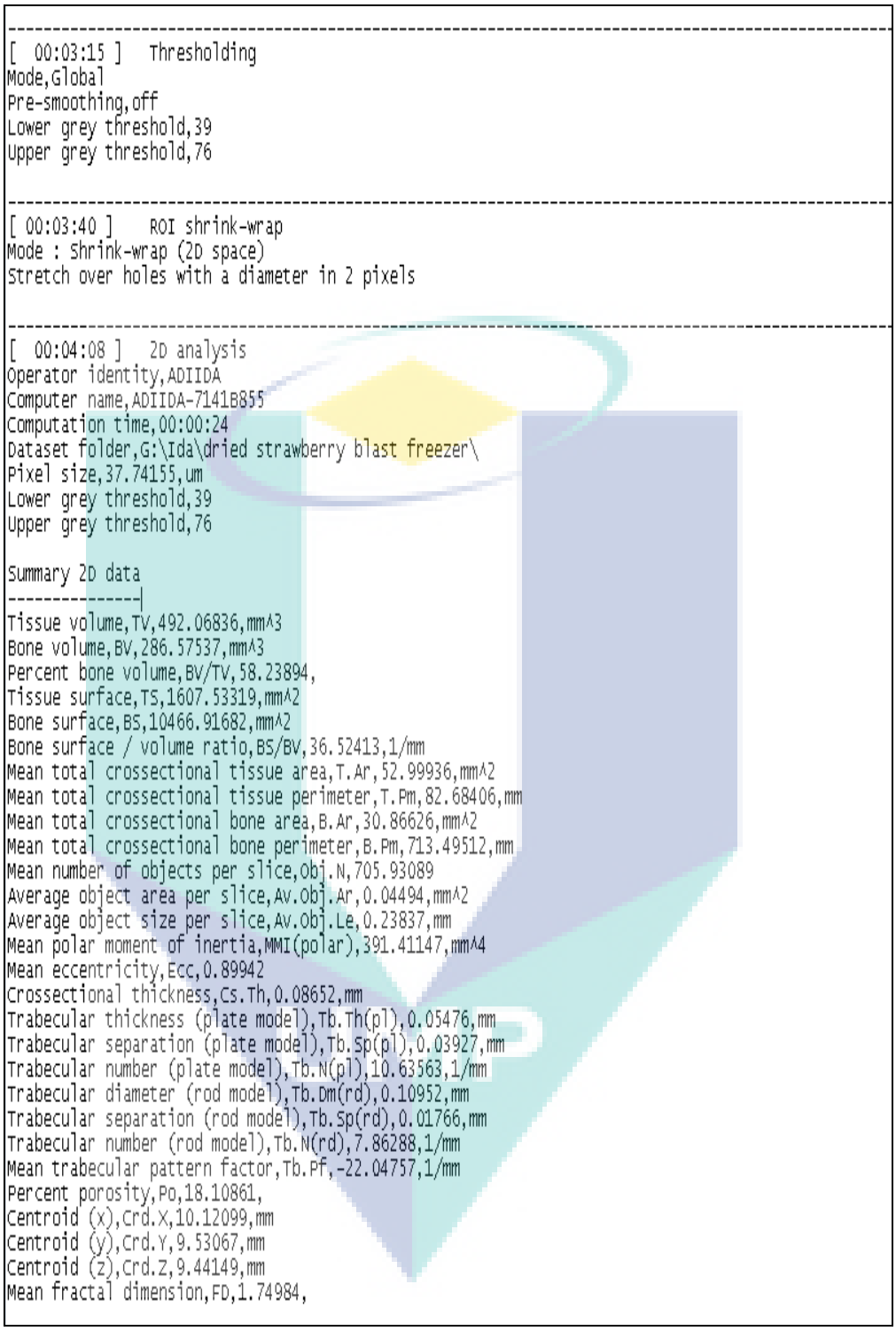


Figure A.15: Image J analysis

APPENDIX B
RESULTS DATA



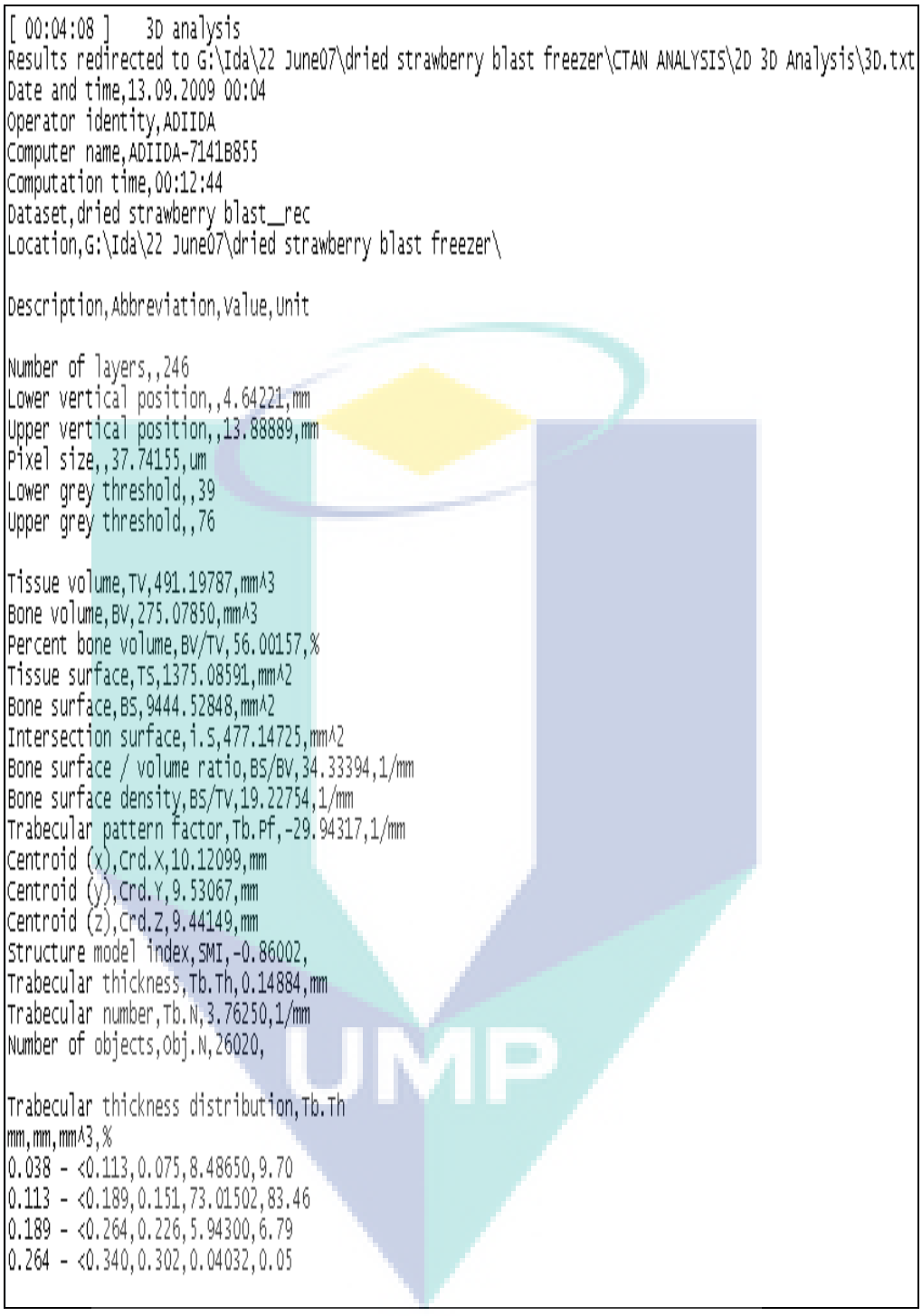
```
[  00:03:15 ]   Thresholding
Mode,Global
Pre-smoothing,off
Lower grey threshold,39
Upper grey threshold,76

[ 00:03:40 ]    ROI shrink-wrap
Mode : Shrink-wrap (2D space)
Stretch over holes with a diameter in 2 pixels

[  00:04:08 ]   2D analysis
Operator identity,ADIIDA
Computer name,ADIIDA-7141B855
Computation time,00:00:24
Dataset folder,G:\Ida\dried strawberry blast freezer\
Pixel size,37.74155,um
Lower grey threshold,39
Upper grey threshold,76

Summary 2D data
---------------
Tissue volume,TV,492.06836,mm^3
Bone volume,BV,286.57537,mm^3
Percent bone volume,BV/TV,58.23894,
Tissue surface,TS,1607.53319,mm^2
Bone surface,BS,10466.91682,mm^2
Bone surface / volume ratio,BS/BV,36.52413,1/mm
Mean total crossectional tissue area,T.Ar,52.99936,mm^2
Mean total crossectional tissue perimeter,T.Pm,82.68406,mm
Mean total crossectional bone area,B.Ar,30.86626,mm^2
Mean total crossectional bone perimeter,B.Pm,713.49512,mm
Mean number of objects per slice,Obj.N,705.93089
Average object area per slice,Av.Obj.Ar,0.04494,mm^2
Average object size per slice,Av.Obj.Le,0.23837,mm
Mean polar moment of inertia,MMI(polar),391.41147,mm^4
Mean eccentricity,Ecc,0.89942
Crossectional thickness,Cs.Th,0.08652,mm
Trabecular thickness (plate model),Tb.Th(pl),0.05476,mm
Trabecular separation (plate model),Tb.Sp(pl),0.03927,mm
Trabecular number (plate model),Tb.N(pl),10.63563,1/mm
Trabecular diameter (rod model),Tb.Dm(rd),0.10952,mm
Trabecular separation (rod model),Tb.Sp(rd),0.01766,mm
Trabecular number (rod model),Tb.N(rd),7.86288,1/mm
Mean trabecular pattern factor,Tb.Pf,-22.04757,1/mm
Percent porosity,Po,18.10861,
Centroid (x),Crd.X,10.12099,mm
Centroid (y),Crd.Y,9.53067,mm
Centroid (z),Crd.Z,9.44149,mm
Mean fractal dimension,FD,1.74984,
```

Figure B.1: Summarization 2-D data analysis



```
[ 00:04:08 ]    3D analysis
Results redirected to G:\Ida\22 June07\dried strawberry blast freezer\CTAN ANALYSIS\2D 3D Analysis\3D.txt
Date and time,13.09.2009 00:04
Operator identity,ADIIDA
Computer name,ADIIDA-7141B855
Computation time,00:12:44
Dataset,dried strawberry blast_rec
Location,G:\Ida\22 June07\dried strawberry blast freezer\

Description,Abbreviation,Value,Unit

Number of layers,,246
Lower vertical position,,4.64221,mm
Upper vertical position,,13.88889,mm
Pixel size,,37.74155,um
Lower grey threshold,,39
Upper grey threshold,,76

Tissue volume,TV,491.19787,mm^3
Bone volume,BV,275.07850,mm^3
Percent bone volume,BV/TV,56.00157,%
Tissue surface,TS,1375.08591,mm^2
Bone surface,BS,9444.52848,mm^2
Intersection surface,i.S,477.14725,mm^2
Bone surface / volume ratio,BS/BV,34.33394,1/mm
Bone surface density,BS/TV,19.22754,1/mm
Trabecular pattern factor,Tb.Pf,-29.94317,1/mm
Centroid (x),Crd.X,10.12099,mm
Centroid (y),Crd.Y,9.53067,mm
Centroid (z),Crd.Z,9.44149,mm
Structure model index,SMI,-0.86002,
Trabecular thickness,Tb.Th,0.14884,mm
Trabecular number,Tb.N,3.76250,1/mm
Number of objects,Obj.N,26020,

Trabecular thickness distribution,Tb.Th
mm,mm,mm^3,%
0.038 - <0.113,0.075,8.48650,9.70
0.113 - <0.189,0.151,73.01502,83.46
0.189 - <0.264,0.226,5.94300,6.79
0.264 - <0.340,0.302,0.04032,0.05
```

Figure B.2: Summarization 3-D data analysis

File Edit Format View Help

```
[System]
Scanner=SKYSCAN1072_3
[Acquisition]
Converted by=NRecon (Version: 1.4)
Conversion time=Jun 22, 2007  13:57:51
Original configuration file=dried strawberry blast__par.txt
Conversion description=Retrieved info. No conversion.
Acquisition time=Friday, June 22, 2009,  12:36(file creation time)
Source Voltage (kV)=50.000000
Source Current (uA)=98.000000
Use 360 Rotation=NO
Rotation Step (deg)=0.900000
Object to Source (mm)=202.000000
Optical Axis (line)=518
Image Pixel Size (um)=18.857750
Rotation Direction=CC
Image Format=TIFF
Depth (bits)=16
[Reconstruction]
Reconstruction Program=NRecon
Program Version=Version: 1.4.4
Program Home Directory=C:\program_\ctan_ctvol
Dataset Origin=SKYSCAN1072_3
Dataset Prefix=dried strawberry blast_
Dataset Directory=F:\Results\Ida\22 June07\dried strawberry blast freezer
Time and Date=Jun 22, 2009  15:36:35
First Section=24
Last Section=998
Reconstruction duration per slice (seconds)=1.777436
Postalignment=5.50
Section to Section Step=1
Sections Count=975
Result File Type=BMP
Result File Header Length (bytes)=1134
Result Image Width (pixels)=1024
Result Image Height (pixels)=1024
Pixel Size (um)=18.87077
Reconstruction Angular Range (deg)=180.00
Use 180+=0
Angular Step (deg)=0.9000
Smoothing=0
Ring Artifact Correction=13
Draw Scales=ON
Object Bigger than FOV=OFF
Reconstruction from ROI=OFF
Size Compression to 1Kx1K=OFF
Beam Hardening Correction (%)=50
CS Static Rotation (deg)=0.0
Mininum for CS to Image Conversion=-0.0002
Maximum for CS to Image Conversion=0.0231
HU Calibration=OFF
BMP LUT=1
Cone-beam Angle Horiz.(deg)=5.470141
Cone-beam Angle Vert.(deg)=5.470141
```

Figure B.3: Summarization ROI data analysis

Table B.1: Raw data for ice crystals width measurement in strawberry for different freezing method.

No.of measurement	-20°C	-80°C
1	0.29	0.16
2	0.29	0.16
3	0.27	0.20
4	0.30	0.23
5	0.28	0.23
6	0.27	0.22
7	0.30	0.20
8	0.28	0.21
9	0.28	0.23
10	0.29	0.25
11	0.32	0.27
12	0.33	0.28
13	0.35	0.29
14	0.35	0.29
15	0.36	0.28
16	0.37	0.28
17	0.37	0.27
18	0.39	0.27
19	0.39	0.26
20	0.40	0.25
21	0.40	0.25
22	0.40	0.25
23	0.39	0.25
24	0.39	0.25
25	0.38	0.25
26	0.38	0.25
27	0.37	0.25
28	0.36	0.25
29	0.36	0.25
30	0.36	0.25
31	0.35	0.25
32	0.36	0.25
33	0.36	0.25
34	0.36	0.25
35	0.36	0.25
36	0.36	0.25
37	0.35	0.24
38	0.36	0.24
39	0.35	0.25
40	0.35	0.24

Table B.2: Raw data for ice crystals width measurement in strawberry for different thickness of the sample.

No.of measurement	5mm	10mm	15mm
1	0.17	0.31	0.10
2	0.12	0.24	0.07
3	0.14	0.18	0.10
4	0.15	0.17	0.11
5	0.15	0.16	0.12
6	0.16	0.16	0.12
7	0.16	0.17	0.11
8	0.16	0.16	0.10
9	0.15	0.15	0.10
10	0.15	0.14	0.10
11	0.15	0.14	0.11
12	0.14	0.13	0.13
13	0.14	0.13	0.14
14	0.14	0.13	0.15
15	0.14	0.12	0.14
16	0.13	0.12	0.13
17	0.13	0.11	0.13
18	0.12	0.11	0.12
19	0.12	0.11	0.13
20	0.12	0.11	0.13
21	0.12	0.10	0.12
22	0.12	0.10	0.12
23	0.12	0.10	0.12
24	0.12	0.10	0.12
25	0.12	0.09	0.11
26	0.11	0.09	0.11
27	0.11	0.09	0.12
28	0.11	0.09	0.11
29	0.11	0.09	0.11
30	0.11	0.09	0.11
31	0.11	0.09	0.11
32	0.11	0.09	0.11
33	0.11	0.09	0.11
34	0.11	0.09	0.11
35	0.11	0.08	0.10
36	0.11	0.09	0.10
37	0.11	0.09	0.10
38	0.11	0.09	0.10
39	0.11	0.09	0.10
40	0.11	0.08	0.10

Table B.3: Moisture analysis of freeze-dried strawberry

Moisture Analysis (%)			
Moisture loss	**{Wet weight(g) -Dried weight (g)}/ Wet weight(g)**		
Samples: Freeze-dried			
Sample A (5mm)	**Wet weight (g)**	**Dried weight (g)**	**Moisture loss(%)**
1	5.062	0.6295	87.6%
2	3.327	0.534	83.9%
Average			85.8%
Sample B (10mm)			
1	6.099	0.5865	90.4%
2	5.346	0.7822	85.4%
Average			87.9%
Sample C (15mm)			
1	10.484	0.7619	92.7%
2	8.062	0.8739	89.2%
Average			90.9%
Sample D (Half)			
1	13.121	1.1762	91.0%
2	14.593	1.3852	90.5%
Average			90.8%
Sample E (Whole)			
1	17.767	2.2597	87.3%
2	18.707	1.9312	89.7%
Average			88.5%
Sample F (One-Quarter)			
1	8.25	0.693	91.6%
2	7.253	0.6079	91.6%
Average			91.6%
Average moisture loss in freeze-dried samples			**89.2%**
	%		
Moisture remain	**10.8**		

Table B.4: Moisture analysis of freeze-dried jackfruit

Moisture Analysis (%)			
Moisture loss	**{Wet weight(g) -Dried weight (g)}/ Wet weight(g)**		
Samples: Freeze-dried			
Sample A (5mm)	**Wet weight (g)**	**Dried weight (g)**	**Moisture loss(%)**
1	2.238	0.8751	60.9%
2	1.86	0.3963	78.7%
Average			69.8%
Sample B (10mm)			
1	3.722	0.946	74.6%
2	12.764	1.8457	85.5%
Average			80.1%
Sample C (15mm)			
1	36.159	7.5288	79.2%
2	44.183	8.7381	80.2%
Average			79.7%
Sample D (Half)			
1	24.164	4.8214	80.0%
2	18.06	3.5719	80.2%
Average			80.1%
Sample E (Whole)			
1	70.185	15.7871	77.5%
2	52.976	10.4742	80.2%
Average			78.9%
Sample F (One-Quarter)			
1	9.767	2.1499	78.0%
2	9.318	2.0963	77.5%
Average			77.7%
Average moisture loss in freeze-dried samples			**77.7%**
	%		
Moisture remain	**22.3**		

Table B.5: Total phenolics analysis

Sample Preparation	Total Phenolics (mg/100g fresh)
Fresh Frozen strawberries	270.5
Freeze-dried strawberries(-20°C/ Ultrasonic treatment)	231
Air-dried strawberries (60°C)	28.7
Fresh frozen Jackfurits	58.5
Freeze-dried jackfruits (-20°C/ Ultrasonic treatment)	40.7
Air-dried jackfruits (60°C)	8.4

Table B.6: Ascorbic acid analysis for strawberry samples

Sample Preparation	Ascorbic acid Concentrations (Triplicate Analysis)
Fresh strawberries	8.66
Freeze-dried strawberries (-20°C)	3.08
Freeze-dried strawberries(-20°C/ Ultrasonic treatment)	3.65
Air-dried strawberries (60°C)	Not detected

Table B.7: Ascorbic acid analysis for jackfruit samples

Sample Preparation	Ascorbic acid Concentrations (Triplicate Analysis)
Fresh Jackfurits	0.013
Freeze-dried jackfruits (-20°C)	0.0048
Freeze-dried jackfruits (-20°C/ Ultrasonic treatment)	0.0093
Air-dried jackfruits (60°C)	Not detected

Figure B.4: Frozen sample before undergo freeze-drying process

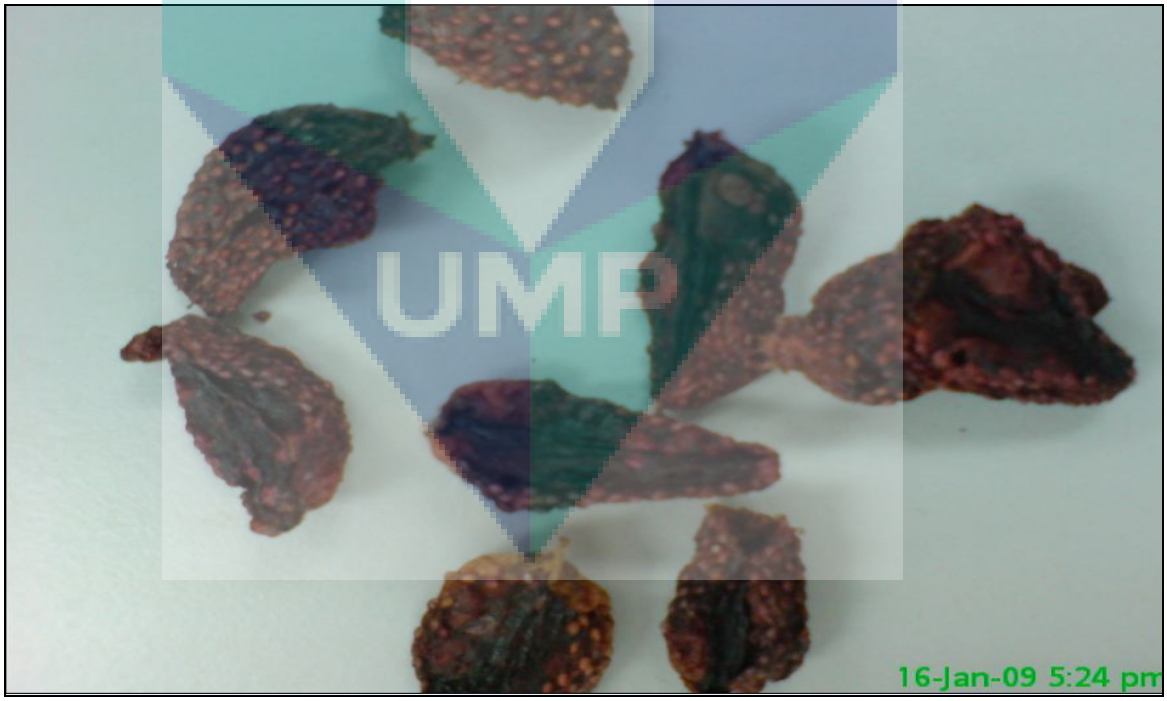


Figure B.5: Freeze-dried strawberry samples

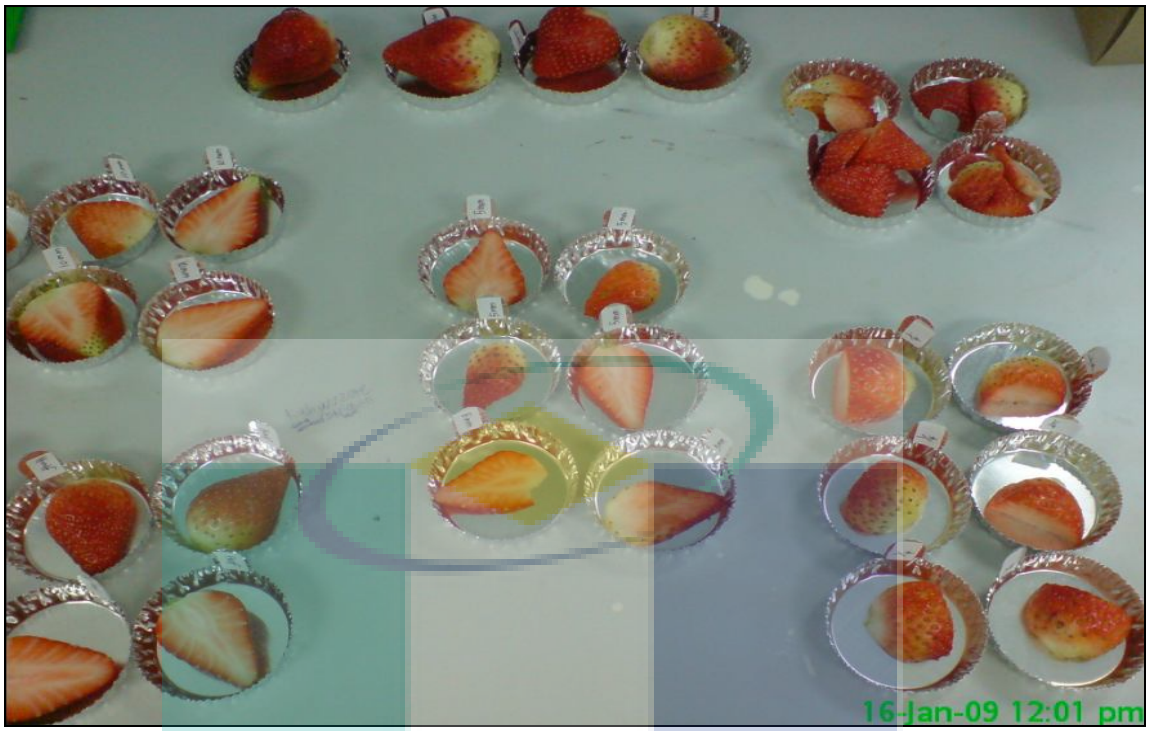


Figure B.6: Various sizes of strawberry samples

Figure B.7: Air-dried strawberry samples

APPENDIX C

LIST OF PUBLICATIONS

LIST OF PUBLICATIONS

C1: Nurzahida, M.Z., Mimi Sakinah, A.M. and Jaafar, A. 2010. Influence of thickness on ice crystal formation in strawberry during freeze-drying. *Journal of Applied Science*. **10 (21):**2741-2744.

C2: Nurzahida, M.Z., Mimi Sakinah, A.M. and Jaafar, A. 2009. A novel technique for ice crystal visualization in fresh strawberry using x-ray micro-computed tomography, Poster presentation - Proceeding of the 8th International Annual Symposium on Sustainability Science and Management(UMTAS), 3rd -5th of May, Primula Resort, Kuala Terengganu, Malaysia, organized by Universiti Malaysia Terengganu.

C3: Nurzahida, M.Z., Mimi Sakinah, A.M. and Jaafar, A. 2009. A novel technique for ice crystal visualization in freeze-dried strawberry and jackfruit using x-ray micro-computed tomography, Oral presentation, Malaysian Technical Universities Conference on Engineering and Technology (MUCEET2009), 20th -22nd of June, MS Garden, Kuantan, Pahang, Malaysia, organized by Universiti Malaysia Pahang.

C4: Nurzahida, M.Z., Mimi Sakinah, A.M. and Jaafar, A. 2009. Influence of thickness on ice crystal formation in strawberry during freeze-drying, Oral presentation, Proceeding of the 3rd International Conference of Chemical and Bioprocessing Engineering Conference and 23rd Symposium of Malaysian Chemical Engineers (ICCBPE-SOMCHE), 12-14th of August, 1 Borneo Novotel, Sabah, Malaysia, organized by Universiti Malaysia Sabah.

C5: Nurzahida, M.Z., Mimi Sakinah, A.M. and Jaafar, A. 2010. Effect of freeze-drying on microstructure of strawberry and jackfruit slices: An illustrative use of X-ray Micro-Computed Tomography in microstructure evaluation of a food product (*submitted for publication in Journal of Food Chemistry).

C6: Nurzahida, M.Z., Mimi Sakinah, A.M. and Jaafar, A. 2010. Effect of ultrasonic assisted extraction on freeze-dried strawberry (*submitted for publication in Journal of Food Engineering).

C7: Nurzahida, M.Z., Mimi Sakinah, A.M. and Jaafar, A. 2010. Drying of fruits using freeze-dried technique: Investigations with strawberry and jackfruit, Abstract accepted, 1st International Conference and Exhibition of Women Engineers, 27-28th of December, Bukit Gambang Resort City, Kuantan, Pahang, Malaysia, organized by Universiti Malaysia Pahang.