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Microstructural changes in strawberry after freezing and thawing processes

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Abstract

The selection of freezing conditions is very important in order to optimize the efficiency of the freezing process and simultaneously retain the biological microstructure. Fresh strawberry and osmotically dehydrated strawberry samples were frozen and thawed using several combinations of temperatures and air velocities. Different freezing and thawing rates were used, which resulted in varying degrees of mass transport from the intercellular to extracellular domain. Scanning electron microscopy (SEM) was used to visualize the degree of structural damages caused, which depended on the crystal size and water movement. SEM showed that better preservation of the structure was obtained using high freezing or thawing rates. When high temperatures were used for thawing, the tissue damage was greater and extensive. Pre-treatment with sucrose reduced the total mass of samples to about 2.5 g/100 g in the osmotically dehydrated samples but did not affect the tissue integrity. It is concluded that the freezing–thawing conditions are responsible for the modifications observed. By using this technique it was possible to establish relationships between the tissue structure and the processing conditions.

Keywords: Freezing–thawing processes; Osmotic dehydration; Scanning electron microscopy (SEM); Strawberry; Structure preservation

1. Introduction

The relationship between the morphological changes that food components undergo during processing and the final textural and sensory characteristics is always of interest for defining the proper conditions that help to retain the quality of the product. Microscopy is being increasingly used to study the influence of processing conditions and ingredients on food structure (Flint, 1994), and only in the last few decades the full potential of electron microscopy (EM) has been recognized (Wilson, 1991). However, the visualization of the true food structure is sometimes difficult, since each step of the preparation of a specimen for microscopy alters the food sample to some extent (Kaláb, Allan-Wojtas, & Miller, 1995). Thus, an important principle in food microscopy is that the less is done to the specimen the better for not altering the structure in great extent (Flint, 1994). SEM is a very useful tool to visualize food structure because in many ways, it combines the best features of the light microscopy (LM) and transmission electron microscopy (TEM) (Aguilera & Stanley, 1999).

Structural research on fruits as affected by processing has been described as time-consuming and tedious because of the large degree of fruit to fruit variability. Visualization of the ''true'' tissue structure is difficult to determine because of the alterations produced during sample preparation for microscopy. The optimal use of microscopy and imaging techniques adapted from the fields of material science, biology and medicine for use on fruit samples is within a multidisciplinary research approach (Alzamora, Gershenson, Vidales & Nieto, 1997). Studies on the structure/processing relationship in edible plant materials have been critically reviewed in the literature (Alzamora et al., 1997). In particular, ultrastructural changes associated with freeze–thaw and blanching have been studied in several types of tissue by Partmann (1975); Bomben and King (1982); Moreno, Chiralt, Escriche and Serra (2000) and Roy, Taylor and Kramer (2001). In general terms all these workers

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described a gradual breakdown in the organization of the protoplasmic structure and, in most cases, the rupture of the plasmalemma with subsequent loss of turgor pressure in cells. In addition, some degradation and separation of cell walls were also noted (Jewell, 1979).

Frozen strawberries have received special attention in recent years. Freezing is a preservation process that may cause severe changes to tissues, resulting in excessive softening. The freezing rate is a variable recognized as responsible for tissue damage (Fuchigami, Kato & Teramoto, 1997) and can result in unacceptable or sub-optimal product characteristics after thawing. It is generally accepted that high freezing rates retain the quality better than slow freezing rates (Partmann, 1975). However, ultrarapid freezing may produce unfavourable effects. Partmann (1975) citing Durif (1971) concluded that the flavour of strawberries was better preserved when they were fast frozen, but Gutschmidt (1969) working with the Senga Sengana variety at optimal ripeness, found that quality could not be improved by using freezing rates greater than 1.5 cm/h. However, it was also shown that for a freezing rate of 0.6 cm/h, the overall quality decreased if the texture and the flavour were both considered (Partmann, 1975). The discrepancies that exist in literature could be due to differences in raw materials, pre-treatment and freezing–thawing conditions.

Strawberries can be pre-treated before freezing by osmotic dehydration that is mixing them with sugars or syrups, because these additives sweeten the product, contribute to retain volatile compounds, reduce the water amount to freeze and decrease the browning by acting as a barrier to oxygen. Furthermore, the water activity is reduced and fruit stability is improved. Osmotic dehydration may cause an exchange of solutes and water, which may cause the shrinking of the cells depending on the solute concentration and its molecular weight.

It is important to adjust the freezing–thawing process variables in order to better preserve and retain the quality of the product. The objectives of this work therefore, were to examine, using scanning electron microscopy (SEM), microstructural changes of fresh strawberries and sucrose–strawberry systems after various freezing and thawing conditions, and to establish relationships between the tissue structure and the processing conditions.

2. Materials and methods

2.1. Sample preparation

Strawberries (*Fragaria x ananassa*) of the Sweet Charlie cultivar were freshly harvested by hand in a local orchard and received in the laboratory. Fruits for processing were selected according to their appearance (ripeness, size and colour). They were washed with tap water, dried with a paper towel to remove undesirable materials and the peduncles removed. Then, they were immediately used, either untreated or treated with sucrose.

2.2. Osmotic dehydration treatment

Granulated sucrose, food grade (100 g/100 g) was used as osmotic medium. Sucrose is usually added in the preservation process for confectionery. Strawberry samples of approximately 2 g were mixed with sucrose by hand in order to obtain a sample/total weight ratio of 25 g/100 g. Samples were handled very carefully so to avoid some structural damage. The osmotic process was held up for 3 h at 20°C in a water-bath. These conditions were chosen after determining experimentally that at 20°C the main gain of solids occurred during the first 3 h as it is observed in Fig. 1, which shows the normalized moisture content (NMC) and normalized solid content (NSC) during osmotic dehydration of strawberry samples (Delgado, 1997). Normalized moisture content and normalized solids content were calculated with respect to initial moisture and solids content:

$$\mathrm{NMC} = \frac{m}{m_0}, \tag{1}$$

$$\mathrm{NSC} = \frac{s}{s_0}, \tag{2}$$

where m_0 and m are the initial and final moisture content in g/g i.d.m. (initial dry matter), and s_0, s is the initial and final mass of solids in the sample, respectively (Lazarides & Mavroudis, 1995). Thorough contact between sugar and fruit was maintained by hand mixing them every 30 min. Then, samples were frozen under different conditions.

2.3. Process conditions

Strawberries samples were placed in a Platinous Rainbow PR-4GM (Tabai Espec Corp., Osaka, Japan) chamber in a holder, which permitted individual freezing or thawing processes. The chamber had a tunnel equipped with a fan, where the sample holder was placed. Natural and forced convection process conditions were tested with air velocities ranging from 0.51 to 4.54 m/s. Table 1 illustrates the combinations of temperatures and air velocities used in the present study. For natural convection, when the tunnel fan was turned off, the air velocity resulting from the chamber air circulation was 0.51 m/s.

Temperature profiles were recorded using T-type thermocouples inserted in the geometric centre of the

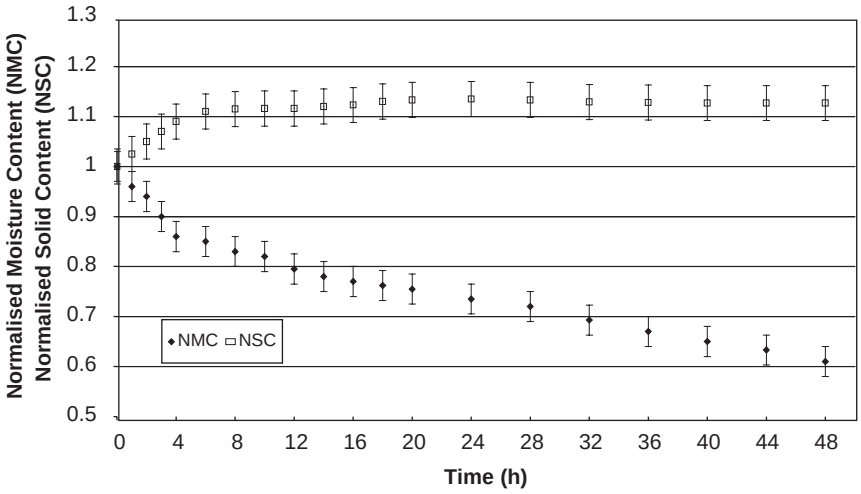


Fig. 1. Normalized moisture and solids content after the osmotic treatment.

Table 1
Freezing and thawing process conditions for fresh and osmotically dehydrated strawberries

Convection	Air conditions	Process	
		Freezing	Thawing
Forced			
	Temperature (°C)	−20/−30	6
	Velocity (m/s)	2.58/3.56/4.54	2.58/3.56/4.54
Natural			
	Temperature (°C)	−20/−30	22

fruit and connected to a data acquisition system (DEC MINC 11).

Freezing was considered complete when the thermal centre reached −18°C, while thawing was considered complete when the final central temperature was −1°C. The influence of the thawing process conditions on the strawberry microstructure was studied on samples frozen at the same temperature and air velocity, −20°C and 2.58 m/s, respectively. By varying temperatures and air velocities (Table 1), freezing or thawing rates from 0.19–2.43°C/min were obtained for the processes separately studied.

Mass and exudate measurements were carried out along with the microstructural observations. Fruit samples were weighed before and immediately after the freezing–thawing processes. For the exudate measurement, samples were left in the chamber after thawing at 6°C and for 3 h. The amount of exudate was obtained by weighing and the results expressed as mass of exudate per 100 g of fruit.

2.4. Scanning electron microscopy

Structural observation was carried out using a JEOL JSM-35 C scanning electron microscope operated at 15 KV. Freeze-drying was the method used for the fresh control and the thawed samples for removing the water prior to the SEM observation. Consequently, for the frozen samples, drying was only necessary since the samples were already frozen. Slices of frozen samples, of approximately 1 cm width and cut transversally to the main axis 0.3 cm thick, were mounted on the metal stubs with silver conducting paint and dried at 0.133 Pa using a laboratory evaporator, VEECO, VE-300 model. Then they were gold coated in the same evaporator at 1500 V and 9.33–12.67 Pa, with coating times ranging from 7 to 10 min according to the characteristics of the equipment. The fresh control and thawed samples were previously frozen by immersion in liquid air (−194.5°C) and dried as before. Finally they were viewed on the SEM. Magnifications of 78×, 240× and 940× were used in most of the micrographs in the present study. Images were captured with a Kodak TMX 120 film. At least two samples for each treatment, which showed similar images, were used for the results.

3. Results and discussion

3.1. Strawberry system

Freezing processes may be roughly classified according to the rate of freezing. Typical rates are, slow freezing, around 0.02–0.2°C/min; commercial freezing, between 0.2–0.83°C/min; and rapid freezing, above 0.83°C/min (Brown, 1991). In this work, strawberry samples were subjected to freezing conditions to obtain freezing rates ranging from slow to fast freezing. Fig. 2 shows a typical temperature-time freezing curve obtained experimentally for a fast freezing rate (2.43°C/min).

The firmness of plant-derived foodstuffs depends on cell turgor. Two pre-requisites for the maintenance of

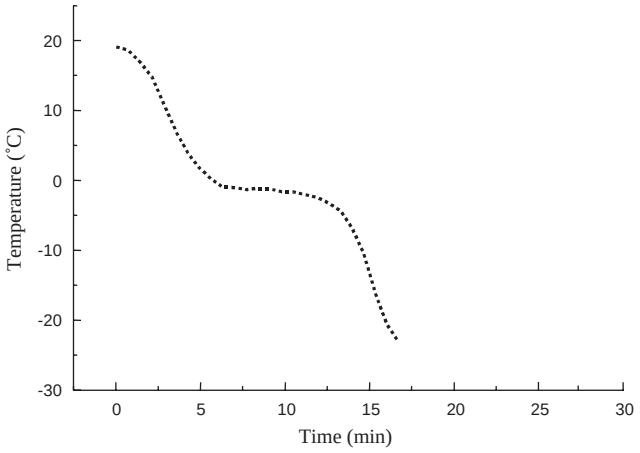


Fig. 2. Characteristic temperature—time freezing curve. Central temperature recorded at 4.54 m/s and −30°C.

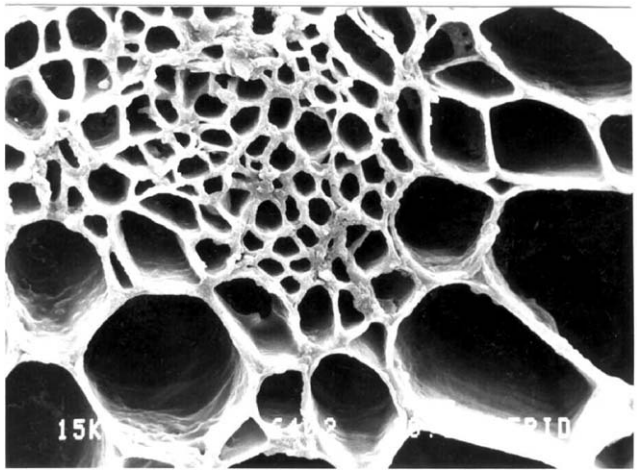


Fig. 4. Scanning electron micrograph of a sample frozen at 4.54 m/s and −30°C (2.43°C/min).

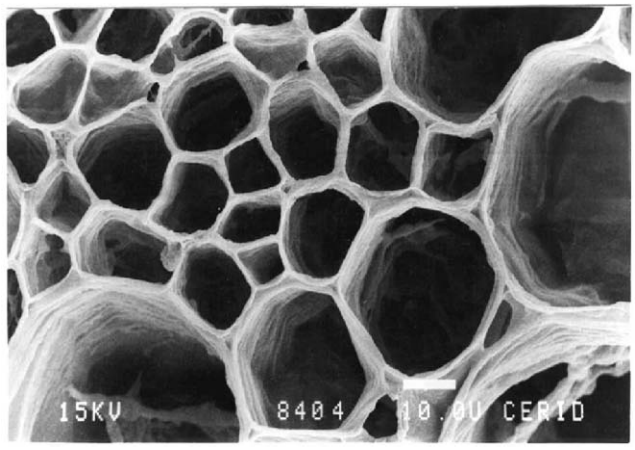


Fig. 3. Cellular structure of fresh strawberry tissue.

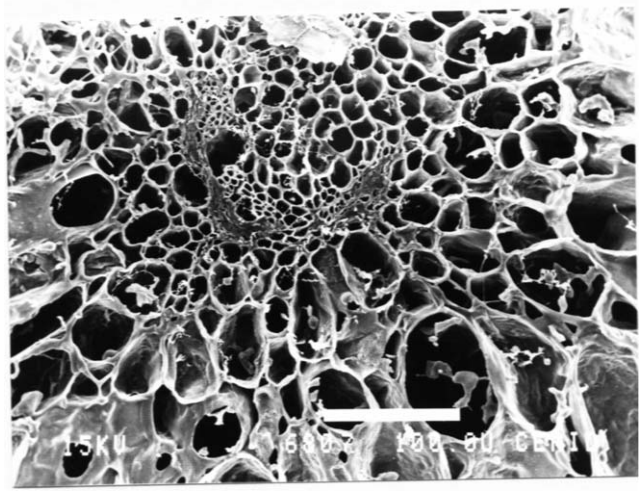


Fig. 5. SEM of parenchymous tissue of a sample frozen at 0.82°C/min.

cell turgor are intact vacuolar and cell membranes to retain osmotic pressure, and intact cell walls to maintain hydrostatic pressure (McLellan, Morris, Grout, & Hughes, 1991). Thus, the direct visualisation of membranes by the use of electron microscopy may be a faster determination of membrane modification (Stanley, 1991). Membranes and their degradation play major roles in food quality and are generally underestimated in their importance in food quality. Fig. 3 shows a control sample of strawberry tissue, which did not receive any other treatment but the preparation for SEM. The bright regions in the micrograph are mainly the cytoplasmic membrane and the cell walls; the darker regions are holes where ice and cell contents were before (Bomben & King, 1982). The integrity of isodiametric cells of parenchymatous tissue with intact membranes, which have not been affected due to the small size of the ice crystals, can be observed. An example of a sample frozen at a fast freezing rate (2.43°C/min) is shown in Fig. 4. The appearance quite similar to that observed in micrograph of Fig. 3 would indicate that the freezing rate was rapid enough that the ice nucleation and crystal growth did not damage the cell walls and ice formation was mainly intracellular.

Fig. 5 shows a SEM micrograph of strawberry tissue frozen at 0.82°C/min which was obtained with an air velocity and ambient temperature equal to 2.58 m/s and −20°C, respectively. Under this operating condition, the cells appeared torn and irregular in shape and some loss of amorphous material and tissue distortion were observed. Hence, there was evidence of extracelluar freezing. Bomben and King (1982) noted that the transition from intracellular to extracellular ice occurred in apple tissue at cooling rates of approximately 1 K/min, which is in the range of the freezing rate applied to the sample of Fig. 5.

Slow freezing rates mainly produce alterations in the transport properties of cell membranes that may result

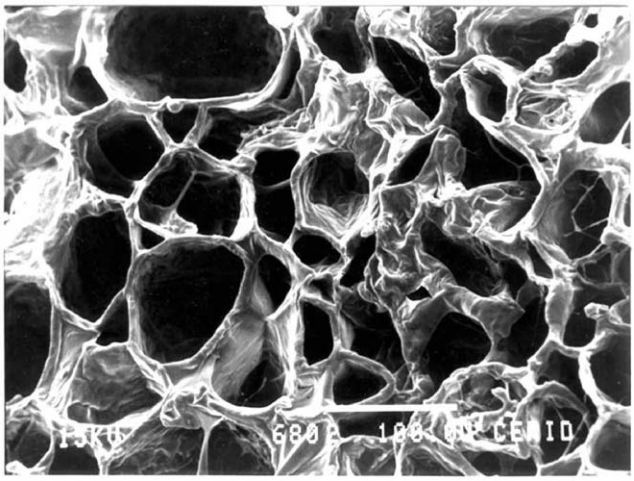


Fig. 6. Strawberry sample frozen at 0.51 m/s and −20°C, note damaged and folded tissue.

from either protein denaturation or alterations in lipid–protein interactions. A consequence of membrane deterioration is the loss of the ability to act as a semi-permeable membrane or diffusion barrier. This has practical consequences in terms of leaching of cellular substances from tissues and water loss, which change thermal properties, flavour and taste (Delgado, 1997). The tissue appearance of Fig. 6 would indicate that extracellular freezing caused the tissue shrinkage and the cell collapse (Delgado & Rubiolo, 1994). It is worth to note that the freezing rate applied to the sample of Fig. 6, 0.34°C/min, is within the low range of what can be considered as commercial freezing, and that structure disruption and folding surfaces could be observed with retention also of whole membranes. The microstructural observations were supported by weight lost measurements, since during freezing some dehydration or water loss occurs. Table 2 shows the variation in weight (Δp) due to evaporation or sublimation. It can be observed that for a fixed temperature, the increase in air velocity resulted in a reduction in weight loss, that is, high freezing rates retained the quality better than slow freezing rates.

Thawed samples, all frozen under the same freezing conditions, were the most structurally damaged as it was expected from their thermal history. Strawberries samples subjected to heating rates of 0.33°C/min and 0.27°C/min are shown in Fig. 7. The thawing operating conditions were 4.54 m/s and 6°C for micrograph **a**, and 0.51 m/s and 22°C for micrograph **b**. It was very difficult to identify individual cells in micrograph **b** due to the wall structural collapse. The freezing-thawing rate is a variable that is function of the size and surface area of the product being frozen-thawed, its thermal conductivity and the gradients of temperature existing within it and the temperature differences at its boundaries

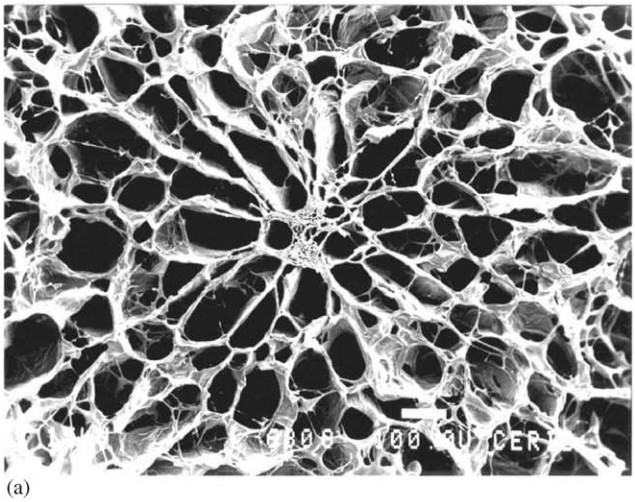
Table 2
Mass lost during freezing

Air velocity (m/s)	Temperature (°C)	$\Delta p/s_0$ (g/g i.d.m.)
0.51	−20	0.109 ± 0.002
2.58	−20	0.058 ± 0.002
3.56	−20	0.052 ± 0.002
0.51	−30	0.105 ± 0.005
2.58	−30	0.077 ± 0.003
4.54	−30	0.072 ± 0.003

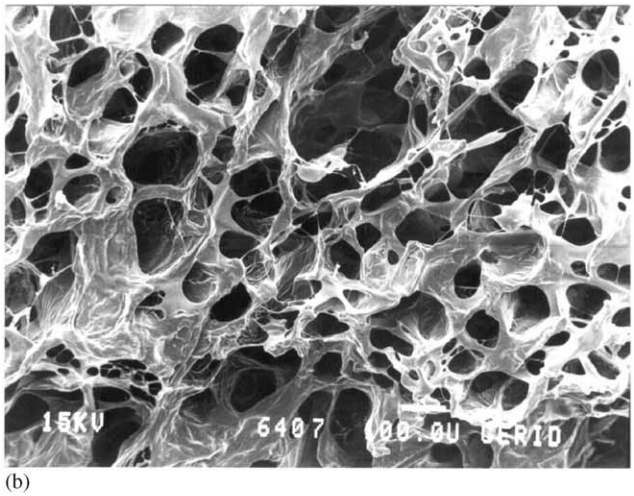
(Brown, 1991). Then, the same heating rate can be obtained by changing any of the above variables. The effect of a similar heating rate, 0.27°C/min obtained by using 2.58 m/s and 6°C is also shown in Fig. 7c. Although, shrunken cells and tissue disruption were observed, the lower ambient temperature and higher air velocity seemed to be less destructive for the strawberry quality than the conditions applied for the experiments shown in micrograph **b**. The results obtained by measuring the exudate volume produced at 0.27°C/min and corresponding to micrographs 7b and 7c were 0.491 g exudate/100 g and 0.096 g exudate/100 g, respectively, which is in agreement with the microscopic observations. Therefore, slow air velocities caused greater tissue damage and also, the higher the ambient temperature the higher the deterioration.

3.2. Strawberry–sucrose system

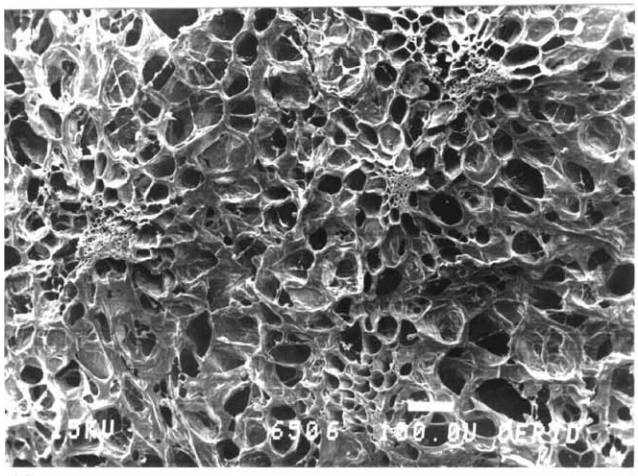
Osmotic processes cause different alteration levels in cells as function of the distance from fruit epidermis, thus defining a structural profile (Moreno et al., 2000). An osmodehydrated strawberry sample frozen at 1.72°C/min and corresponded to an area near to the surface is shown in Fig. 8. The zone of small cells represents conducting tissue. The tissue presented integrity except in certain areas where the shrinkage of some small cells was observed. As a result of the osmotic dehydration treatment the sucrose passes through the cell wall and accumulates between the cell wall and the cellular membrane, where it forms an hypertonic solution leading to a water outflux through the cellular membrane (Mascheroni & Spiazzi, 1997). As a consequence of this exchange, the product will lose more or less weight and eventually it will shrink depending on the solute concentration and its molecular weight for a defined period of time. High solute concentration solutions and high molecular weights favour water loss (Mascheroni & Spiazzi, 1997). Samples of Figs. 8 and 4 were both subjected to rapid freezing rates and appeared to be rather similar though the sucrose pre-treatment. Thus, it could be said that under the operating conditions employed, the previous treatment with sugar that resulted in a 2.5 g weight loss/100 g, did not change greatly the tissue structure; and ultrastructural



(a)



(b)



(c)

Fig. 7. Scanning electron micrographs of parenchymous tissue of samples thawed at different operating conditions: (a) 4.54 m/s and 6°C (0.33°C/min); (b) 0.51 m/s and 22°C (0.27°C/min) and (c) 2.58 m/s and 6°C (0.27°C/min).

differences observed in the untreated sample, could be mainly due to the lower freezing time of the sample shown in Fig. 4.

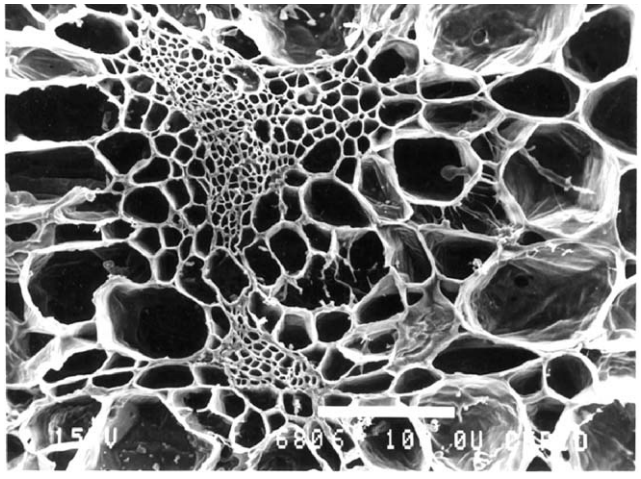


Fig. 8. SEM micrograph of an osmotic pre-concentrated sample with sucrose, frozen at 4.54 m/s and −30°C (1.72°C/min).

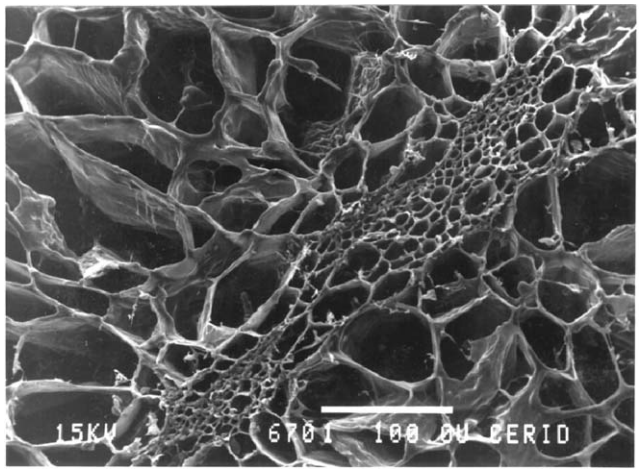


Fig. 9. Sucrose-strawberry sample frozen at 2.58 m/s and −20°C (0.92°C/min).

The SEM micrograph of a sample frozen at 2.58 m/s and −20°C is shown in Fig. 9. The integrity of the tissue was lost in certain areas. When comparing with the SEM micrograph of Fig. 5, that is, with a sample subjected to the same freezing conditions, the similar appearance of both samples would evidence that the sucrose pre-concentration on the surface did not greatly modify the tissue structure.

The positive effect of rapid freezing rates due to low temperatures is depicted in the micrograph of Fig. 10, which was processed at 2.58 m/s and −30°C. The integrity preservation of cell walls was better maintained under this freezing rate, 1.52°C/min. This result is in agreement with the weight lost values obtained for micrographs 9 and 10, which was 0.096 and 0.065 g/g i.d.m, respectively.

The loss of cell compartmentalization and cell walls severely damaged are observed in the SEM micrograph of Fig. 11, after applying a heating rate of 0.19°C/min

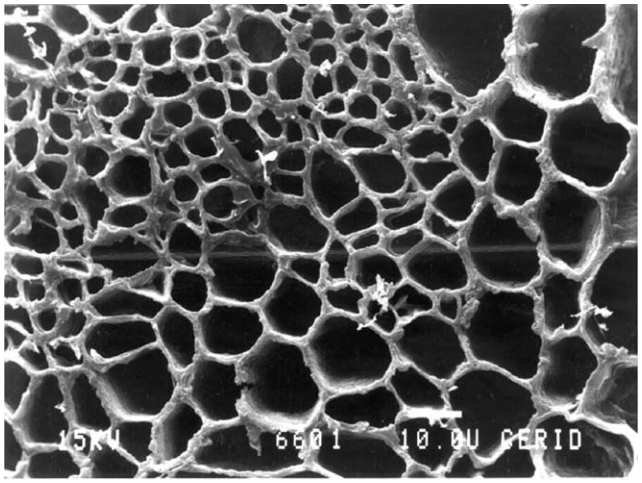


Fig. 10. Scanning electron micrographs of a sample treated with sucrose and subjected to 2.58 m/s and −30°C (1.52°C/min).

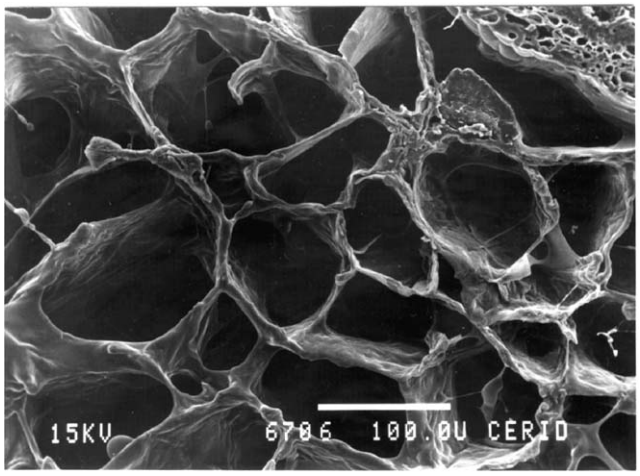


Fig. 12. Tissue thawed at 4.54 m/s and 6°C (0.26°C/min) showing tissue disruption.

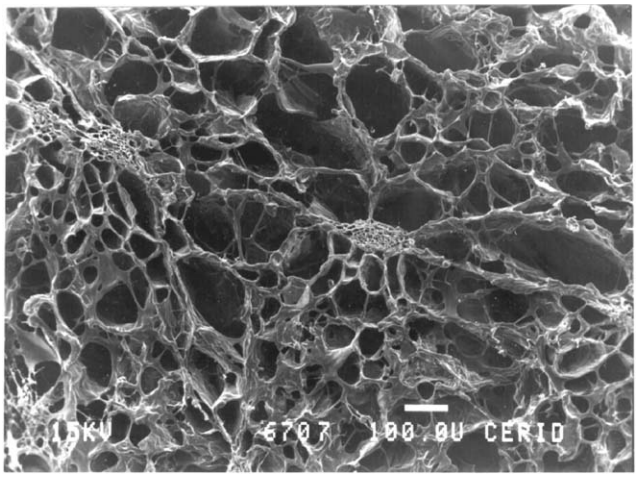


Fig. 11. Micrograph showing cellular collapse of a sucrose–strawberry sample thawed at 2.58 m/s and 6°C (0.19°C/min).

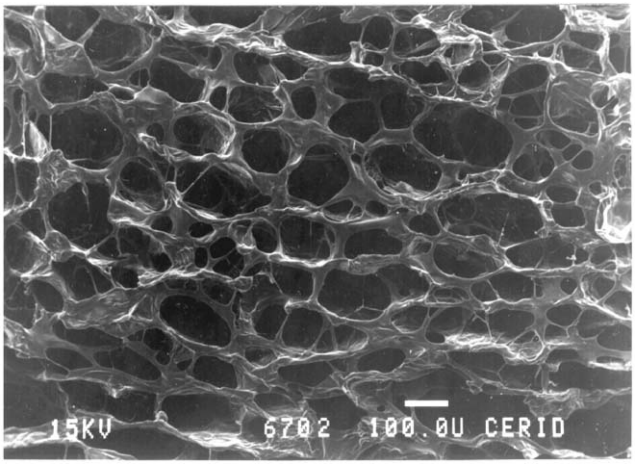


Fig. 13. Sample thawed at 0.51 m/s and 22°C (0.40°C/min) showing cell wall deteriation.

during thawing. A higher air velocity, 4.54 m/s instead of 2.58 m/s (Fig. 11), allowed to preserve the membrane systems slightly better at 6°C as it can be seen in Fig. 12. Nevertheless, both heating rates were quite slow to avoid the tissue shrinkage in samples of Figs. 11 and 12. When an ambient temperature of 22°C was used, higher tissue degradation was observed in spite of the higher thawing rate applied to the sample and equal to 0.40°C/min, which could be attributed to the high ambient temperature (Fig. 13).

4. Conclusions

It was observed from the process conditions considered in this study, that samples which had undergone to low ambient temperatures or high air velocities, i.e. rapid freezing rates (short freezing times), presented the better structure preservation. Freezing rates higher than 1.5°C/min appeared to be appropriate to retain the tissue structure. Membrane degradations or the damage caused as a result of inappropriate freezing conditions cannot be improved during thawing by using high heating rates. However, to reduce water loss or exudate and maintain the tissue integrity to some extent, high heating rates were more convenient, which also reduced the thawing times. Osmotically dehydrated samples with the sucrose concentration used did not greatly affect the tissue structure, and it was the thermal process itself that affected the system. Finally, the freeze-drying preparation method when applied or the drying procedure used, proved to be good since it required a minimal sample manipulation and thus the damage caused to samples during SEM preparation could be reduced.

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