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Laura Otero · María T. Solas · Pedro D. Sanz
Carlos de Elvira · Juan A. Carrasco

Contrasting effects of high-pressure-assisted freezing and conventional air-freezing on eggplant tissue microstructure

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Abstract Damage to the microstructure of eggplants frozen by conventional air-freezing methods and by a high-pressure-assisted freezing method is compared in this paper. When conventional air-freezing techniques are employed, damage to the microstructure is enhanced as the freezing rate diminishes and the sample volume increases. However, when high-pressure-assisted freezing is applied, bulk nucleation occurs simultaneously in the whole sample, and the observed damage is less dependent on the volume of the sample.

Key words High pressure · Freezing · Eggplant

Introduction

Use of an adequate freezing method allows fresh fruits and vegetables to conserve all their organoleptic characteristics (flavour, colour, aroma, etc.) and their nutritional value [1].

Nevertheless, mainly due to the size and location of ice crystals, severe damage can be caused to tissues; this affects the texture and leads to the production of large drips during thawing.

The crystallisation of ice is achieved in two steps: the uniform formation of nuclei and the subsequent growth of these to a specific size. The temperature of the product to be frozen must decrease below the solid-liquid equilibrium point to produce nucleation. The number of nuclei created depends on the extent of supercooling; for each degree K of supercooling the ice-nucleation rate increases ca. tenfold [2]. The crystals only grow when nucleation has taken place, and the size of the ice crystals is a function of the rate of both nucleation and crystalline growth.

L. Otero · P.D. Sanz (✉) · C. de Elvira · J.A. Carrasco
Instituto del Frío (CSIC), Ciudad Universitaria, E-28040 Madrid, Spain

M.T. Solas
Departamento de Biología Celular, Universidad Complutense,
E-28040 Madrid, Spain

Many workers have shown that the freezing rate is a major factor causing cell disruption [3–9]. The freezing rate is defined as the ratio of the minimal distance between the surfaces of the sample and its thermal centre/nominal freezing time, i.e. the time elapsed between the surface reaching 0 °C and the thermal centre reaching a temperature 10 °C lower than that which occurs when ice initially forms at this point [10].

In slow freezing, as the temperature decreases, the water vapour contained in the intercellular spaces condenses and is then transformed into ice. As the extracellular ice crystals produced have a vapour pressure lower than that of water in the cells, the pressure difference leads to the loss of water molecules from the cells which aggregate to form extracellular ice crystals. Slow freezing rates provide the time for water to leave the cells by permeation through the membranes, and thereby keep the intracellular solution near the solute concentration that has an equilibrium freezing point at the prevailing temperature. Ice in the intercellular spaces forces the cells apart, compressing cells and tearing across cell walls [3]. Many micrographs of ice formation in fruit and vegetables are available, and they show the existence of large ice structures, shrunken cells and ruptured cell walls when the freezing rate is low. It is not known whether the tearing across cell walls is due to penetration by growing ice crystals or whether it is secondarily induced by tensile forces caused by expansion of ice crystals in nearby intercellular spaces [3].

If the cell is cooled rapidly, water cannot permeate rapidly enough; thus, the concentration of the intracellular solution departs markedly from its equilibrium, and the probability of intracellular ice nucleation is increased [11]. Ice forms within the cell and plasmolysis does not occur. Available micrographs show cells with the appearance of those found in fresh tissues and no evidence of damage to their walls.

However, when large-volume foods are frozen it is difficult to reach high freezing rates in the whole sample. The larger the volume, the lower the freezing rate at the thermal centre of the sample due to thermal gradients established between the surface and the centre of the

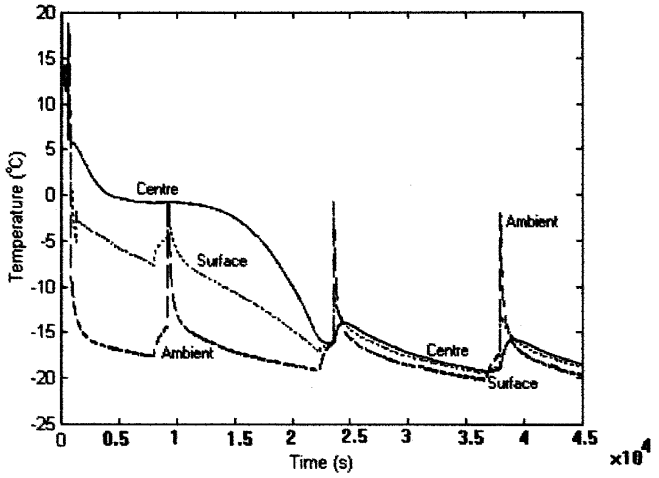


Fig. 1 Temperature curve of eggplant tissue subjected to still-air freezing

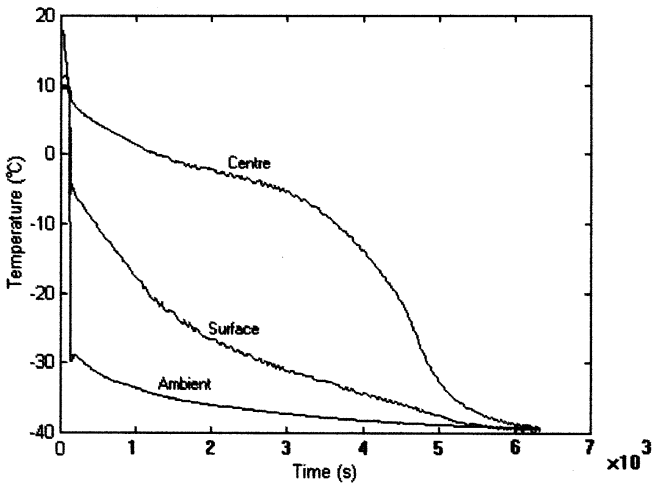


Fig. 2 Temperature curve of eggplant tissue subjected to air-blast freezing

product. Eggplant is a vegetable with a relatively large volume, so, when it is frozen, thermal gradients appear and the freezing rate at the centre is low.

In order to reach high freezing rates at the centre of samples, cryogenic media must be employed, but a higher freezing rate does not always mean that the quality of the final product is better. Exposure to cryogenic media may lead to cracking or shattering of frozen food; this damage, due to the expansion associated with the transition from the water to the ice phase, and internal stresses, is critical and irreversible.

The objectives of this paper are to evaluate the damage to the microstructure and texture of eggplants, and drip losses after thawing, caused by the use of conventional freezing methods with diverse freezing rates and to compare these with the damage and drip losses caused by the new high-pressure-assisted freezing method.

Materials and methods

Mature eggplants with an average diameter of 7 cm and an average length of 18 cm were bought in a local market and packed under vacuum using BB4L polyethylene bags from Cryobag. This vegetable was chosen because of its relatively large volume and high moisture content, i.e. 92.7% [12].

Two kinds of air-freezing methods have been used: still-air freezing and air-blast freezing. Although the former is not an adequate method nor commercially recommended, it was chosen to show the effect of a method with a low freezing rate. In order to avoid freeze-cracking, cryogenic media were not employed. The temperature of the chamber in the still-air-freezing method was –20 °C. The air temperature in the air-blast-freezing method was –40 °C and the air velocity was 5.5 m/s.

High-pressure-assisted freezing was performed using equipment from ACB GEC Alsthon, with a 2.35-l vessel complemented with a thermally isolated thermostatic circuit and a computerised system for the measurement of pressure and temperature. The pressure was supplied by means of two hydro-pneumatic pumps with an ethylene glycol/water solution (1:3, v/v) as the compressing fluid.

Two thermocouples were disposed in each sample(on the surface and in the centre) to track the rate of freezing. Data were recorded at intervals of 15 s or less by means of a computerized system situated about 2 m from the experimental devices.

The microstructure was examined by conventional scanning electron microscopy (SEM). The samples were cut, immediately after freezing, at ambient temperature, fixed in 20% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 and stored for a minimum of 72 h at 3 °C. The samples were then post-fixed with Os O_4, washed, dehydrated in acetone, critical-point dried, sputter-coated with gold/palladium and scanned at 20 kV by SEM (JEOL JSM 6400, Japan). The texture was evaluated by a puncture test using a cylindrical 5-mm-diameter plunger attached to an Instron machine, model 1140, equipped with a 50-N load cell. Samples were punctured through its large axis. Values for rupture strain (%) and firmness (N/m) were determined from the force-distance curves obtained.

The drip losses (%) after thawing were calculated as the weight difference of eggplants before freezing and after thawing at ambient temperature during one night.

Analysis of variance together with an *F*-test and Duncan's multiple range test were used to compare means and to identify significant differences ($P < 0.05$) among treatments.

Results and discussion

Freezing processes

Freezing processes were considered to be completed at –18 °C. The data describing freezing processes using still air and air-blast are presented in Figs. 1 and 2. The three recorded oscillations in Fig. 1 of the ambient temperature correspond to automatic defrosting in the chamber. These oscillations markedly affected the temperature at the surface of the sample and their effects were also observed at the centre when it reached a sufficiently temperature. The average freezing rate was 2.2×10^{-6} m/s. In Fig. 2 ambient, surface and centre temperatures are also recorded. In this faster process, where the freezing rate was 9.4×10^{-6} m/s, the final temperature was reached earlier than in the previous process.

The evolution of pressure and temperature in high-pressure-assisted freezing is shown in Fig. 3. The curve A-B corresponds to the establishment of initial, stable

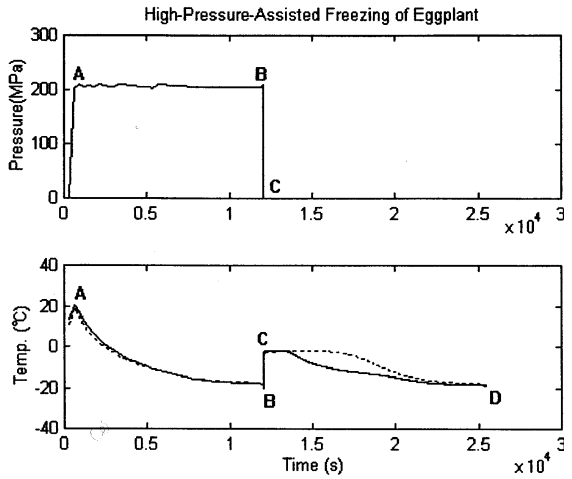


Fig. 3 Freezing of eggplant tissue in relation to pressure and temperature during high-pressure-assisted freezing

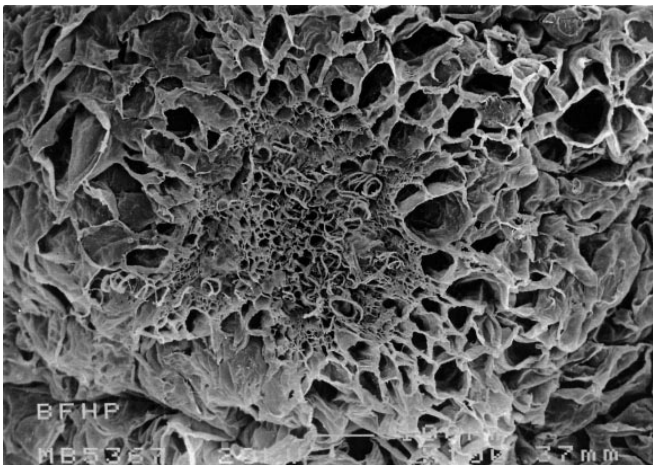


Fig. 4 Cells of the parenchyma of fresh eggplants

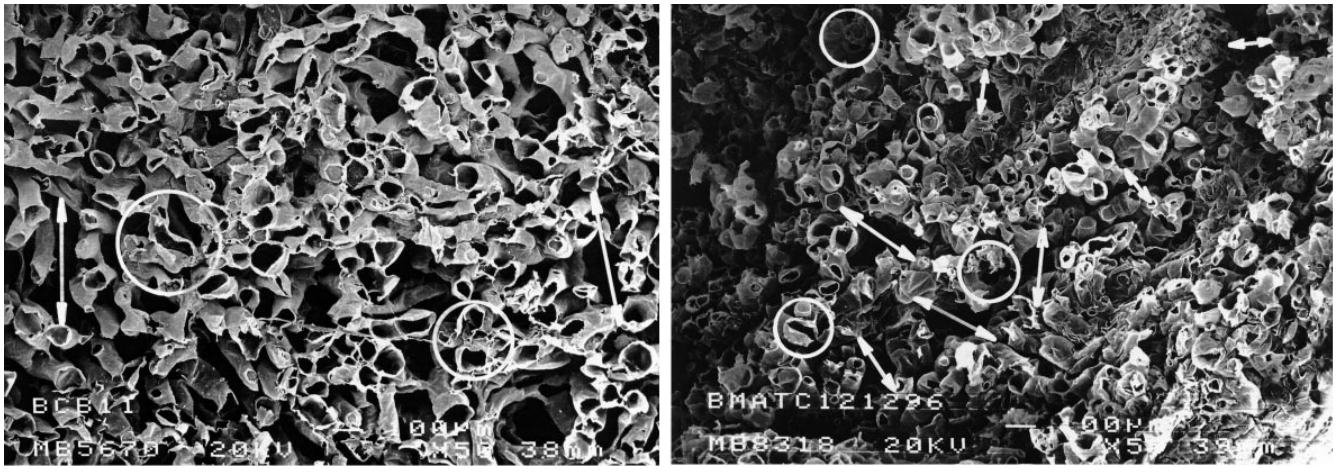


Fig. 5 Surface (*left*) and central (*right*) regions of still-air-frozen eggplant tissue

conditions, prior to freezing at 200 MPa and –20 °C [13]. At B, a quasiadiabatic expansion occurred, yielding a high level of supercooling of about 20 °C and a massive nucleation in the entire sample, due to the isostatic pressure. After this, the ice crystals grew at atmospheric pressure until they attained their final crystal size.

The last part of the process (curve C-D) can be described as classic freezing at atmospheric pressure. It can occur in the vessel of the high pressure device or in another classic freezing device with a high heat extraction rate. The time required for the freezing process to finish depends on the size of the sample, the heat extraction rate, etc. Operating with high-pressure-assisted freezing methods, freezing rates of up to 2.7×10^{-5} m/s were registered in the high-pressure vessel; these values were evaluated following the definition of the International Institute of Refrigeration, which is not quite correct since the initial temperatures (after expansion, point C in Fig. 3) at the surface and in the centre were lower than 0 °C.

A different method, employing freezing at atmospheric pressure in a high pressure vessel under similar conditions (into the same pressure-transferring medium and establishing the same thermal gradients) was not considered because it is not used commercially; neither is it recommended as it produces lower freezing rates than air-freezing [14].

Effects on the cellular structure

Histological changes of fresh and frozen eggplants treated by still air, air-blast and high-pressure-assisted freezing methods are compared in Figs. 4–7. Figure 4 shows cells of the parenchyma of fresh eggplant with cell walls well defined. The cells are arranged together and surrounded by small intercellular spaces. In the centre of the micrograph a relatively isolated vascular bundle can be seen.

Figure 5 shows micrographs of eggplant frozen by the still-air-freezing method (lowest freezing rate) corresponding to the surface and centre, to the left and right, respectively. Cell separation, denoted by arrows, is very clear in both micrographs, and occurred to a larger degree in the centre than on the surface due to thermal gradients. Cell

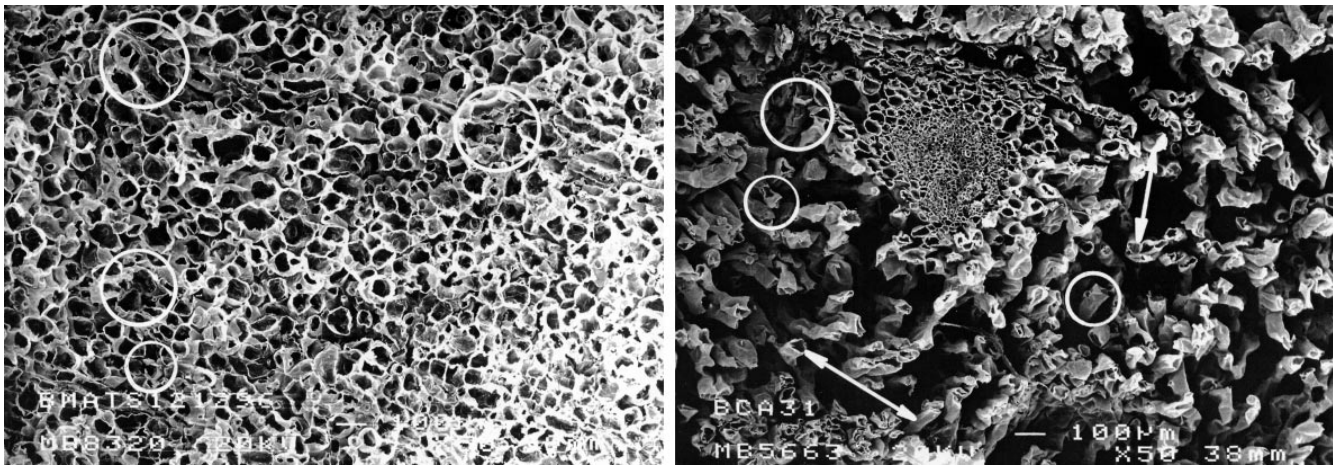


Fig. 6 Surface (*left*) and central (*right*) regions of air-blast-frozen eggplant tissue

walls which were disrupted, characteristic of low freezing rates, are marked with circles and can be clearly seen in both micrographs.

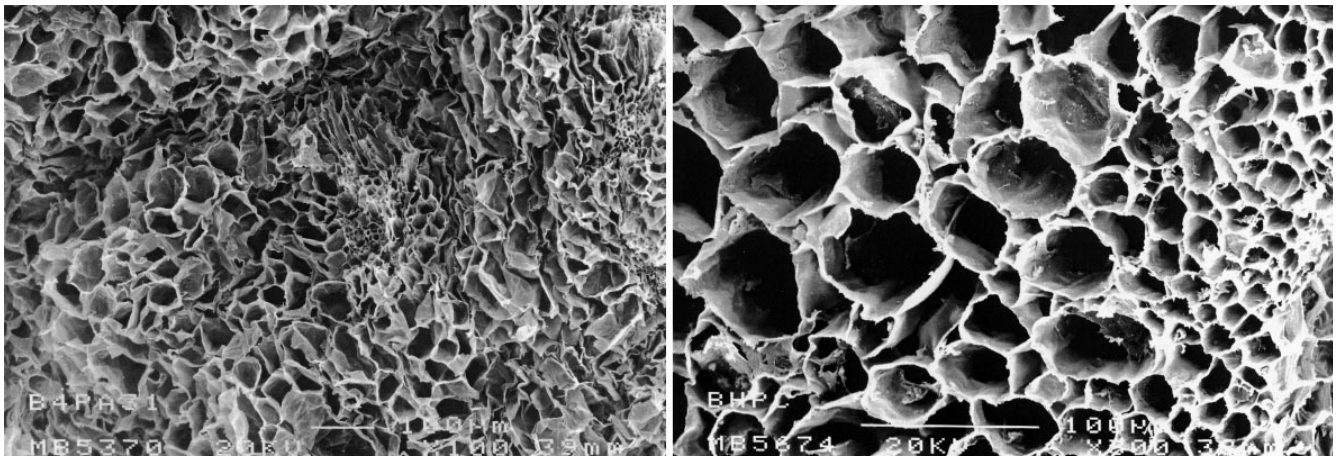
Figure 6 shows micrographs of the surface and centre of an eggplant frozen by air-blast. At the surface (left), the cells appear together with no large gaps between them. However, some broken cell walls can be seen. In general, cell damage is slight. On the right, cell separation and damage are evident, due to the lower freezing rate than that at the cell surface.

Figure 7, on the left, shows the central region of an eggplant frozen by high-pressure-assisted freezing. It has the appearance of a fresh sample. All the cells are positioned together and no cellular damage is evident. There are no substantial differences between the surface and the central region of the sample because nucleation occurred in both at the same time. This is the great advantage of high-pressure-assisted freezing techniques: there is no time for water translocation and ice is formed intracelullarly. Figure 7, on the right, is a magnification of the micrograph on the left. It confirms that there was no damage to the cell walls.

Effect on the texture

The experiments were repeated four times. The results always show the same effect of the treatments on texture and drip losses, but the data for each replicate differ depending on the maturaty of the eggplants employed. The results presented in this paper correspond to those of one of the experiments in which each treatment was replicated three times. Fresh eggplants disintegrated when they were compressed to ~20% of their thickness (Fig. 8). In frozen eggplants the rupture strain is much bigger; in descending order of magnitude: still-air freezing (~75%) $>$ air-blast freezing (~69%) $>$ high-pressure-assisted freezing (~56%). Significant differences were found among all the treatments (Duncan's multiple range test, $P < 0.05$). Firmness (N/m) was calculated from the initial slope of the force-distance curves. The results show how freezing always causes a decrease in firmness (Fig. 9). This decrease in firmness is significantly higher when conventional air-freezing is used in comparision to high-pressure-assisted freezing.

Fig. 7 Central region (*left*) of high-pressure-assisted frozen eggplant tissue and a magnification (*right*)



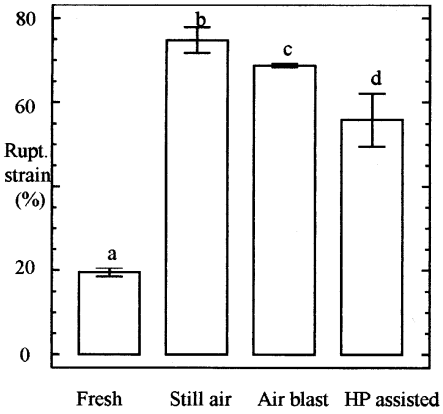


Fig. 8 Rupture strain (%) in eggplants. Mean ± standard deviation. Different letters indicate significant differences (Duncan's multiple range test, $P < 0.05$). *HP* High pressure

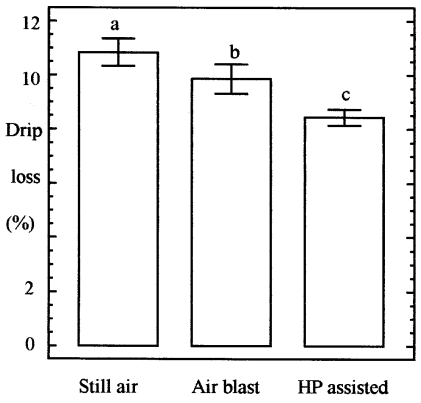


Fig. 10 Drip losses in thawed eggplants. Mean ± standard deviation. Different letters indicate significant differences (Duncan's multiple range test, $P < 0.05$). For abbreviation, see Fig. 8

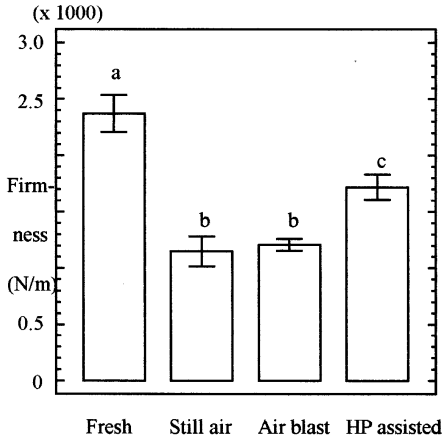


Fig. 9 Firmness (N/m) in eggplants. Mean ± standard deviation. Different letters indicate significant differences (Duncan's multiple range test, $P < 0.05$). For abbreviation, see Fig. 8

Effect on drip losses

Figure 10 shows the effect of the different freezing methods on the drip losses of thawed eggplants. There were significant differences among all the methods. Conventional air-freezing methods produced the most highest losses, and losses increased when freezing rates were low.

High-pressure-assisted freezing is effective in maintaining the structure of frozen eggplants in relation to conventional still air and air-blast freezing methods. This method leads to less textural damage and lower drip losses. The main advantage of this new freezing method is that a high level of supercooling is reached simultaneously in the entire sample, and consequently a massive nucleation is performed, thus avoiding the disadvantages associated with thermal gradients. So, this technique is of interest as an improved method for freezing relatively large, fresh foods.

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