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Isochoric freezing: A novel technology

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Abstract
According to data compiled by the Food and Agriculture Organization (FAO), up to one-third of all food spoils or is wasted before it is consumed. This amounts to a waste of the land, labor, water, energy, and other resources used in the food's production. Fruits and vegetables have the greatest food waste rates of 40–50% out of all the food types. When it comes to maintaining the quality and safety of perishable goods and prolonging their shelf life, preservation technologies are crucial. One of the primary methods for long-term preservation and storage is freezing at atmospheric pressure. Senescence, enzymatic decay, chemical decay, and microbiological development are among the deterioration processes that are slowed down by freezing temperatures and water crystallization during freezing. But because of ice crystal formation, concentration of solutes, and dehydration of cells, freezing can injure biological tissues chemically and physically. Constant volume (isochoric) preservation is a simple and less labor-intensive alternative to classic cryopreservation techniques. Food products can be kept at subfreezing temperatures through isochoric freezing, which prevents ice from forming inside the products. Comprising a constant volume chamber that can withstand the pressures that build up during processing, the isochoric freezing system also includes a cooling system Because the lsatent heat of fusion reduces with temperature and because the total frozen mass is reduced, this method has the potential to cut freezing energy usage by up to 70%. The isochoric method of preservation holds significant potential as a cryopreservation technique in the field of cryobiology, and it is a useful tool for assessing the thermodynamic properties of cryoprotectants.

Keywords: Isochoric freezing, temperature, pressure, cryoprotectants

Introduction
Food loss occurs every year to the tune of 1.3 billion tonnes, or 33 percent of global production, according to the FAO. Through 2050, food consumption is expected to rise steadily to between 150 and 170% of present levels. Comparatively, all other food items such meat, oil seeds, milk, cereals, fish, and shellfish have lower loss rates than fruits and vegetables (FV). During the postharvest life cycle, there is a greater loss of fruit variabilities (FVs) during the storage stage (10%) compared to the stages of harvesting, saving, processing, and distribution. Conventional freezing methods rely on the isobaric principle, which synchronizes changes in temperature and volume. An infinite amount of the food's fluid is frozen by the isobaric mechanism. Food's cell structure is harmed when ice crystals form inside of it [1]. One of the earliest techniques for preserving food is freezing it. Typically, the conventional freezing technique involves lowering the meal's temperature to -18 °C or lower. As isochoric preservation requires less cryoprotectant and is easier to administer, it is an alternative to traditional cryopreservation techniques. Heat transfer causes the meal's temperature to drop when it comes into touch with the freezing media. System designs are one of the many aspects that affect food quality [2]. The food's temperature drops with heat transfer when it is exposed to the freezing medium. Food quality is influenced by a number of factors, including freezing times, phase changes and crystallization of ice, system designs, and others. Other than the use of incorrect temperature ranges or freezing conditions, which can have significantly negative effects, the freezing process is related with a number of obstacles, such as non-uniformity in freezing rates and expenses involved. There are typically five steps involved in the freezing of food [3]. Food is preserved by freezing, a process that entails bringing a product down to a temperature where ice crystals begin to form inside the product's structure.

Lowering the product's temperature as much as is practically feasible is the process's aim in order to reduce reaction rates that cause a decline in product quality. Food that has been frozen can be kept at a low temperature, which lowers water activity and inhibits the formation of bacteria. But when ice crystals are big, plentiful, and dispersed unevenly, it can cause long-term food harm. Consequently, throughout the production and storage of frozen food, ice growth is an essential component that needs to be watched carefully [4].

Thermodynamics process: This process involves the transfer of heat into and out of the system. It is of 4 types –

- Isobaric process in which pressure remains constant throughout the system.
- Isothermal process in which temperature remains constant in the system.
- Adibatic process is that process in which no heat is exchanged in the system.
- Isochoric process in which volume remains constant in the system.

Thermodynamics of Isochoric Systems

Rubinsky and his fellow researchers in 2005 were first studied the thermodynamic principles of isochoric preservation [5]. Isochoric freezing (IF) is a technologically straightforward method of lowering metabolic processes while preventing the development of ice in the stored food product. It does this by using restricted aqueous thermodynamics to passively generate high pressures at moderate sub-freezing temperatures [6]. The method is to fill a rigid container with a food product and an aqueous solution, freeze the container, and then let the ice to expand, passively pressurizing the system's interior [1]. As per the principle of Le Chatelier, an increase in pressure prevents additional freezing, resulting in a pressured two-phase liquid–ice equilibrium that maintains a stable ice fraction at any sub-freezing temperature. As the system's temperature drops, the system keeps freezing and builds pressure until it hits the triple point of ice 1 h, ice III, and liquid. This point is reached by pure water at about -21 °C and 210 MPa, when about 55% of the volume is turned into ice. Food items can be placed in the liquid portion of the chamber thanks to this regulated freezing process, which also prevents the production of ice crystals and the ensuing biophysical damage [7].

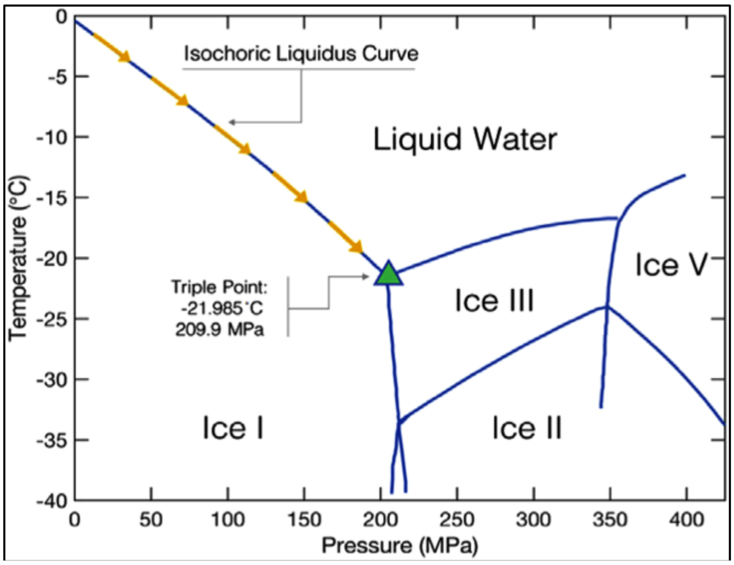
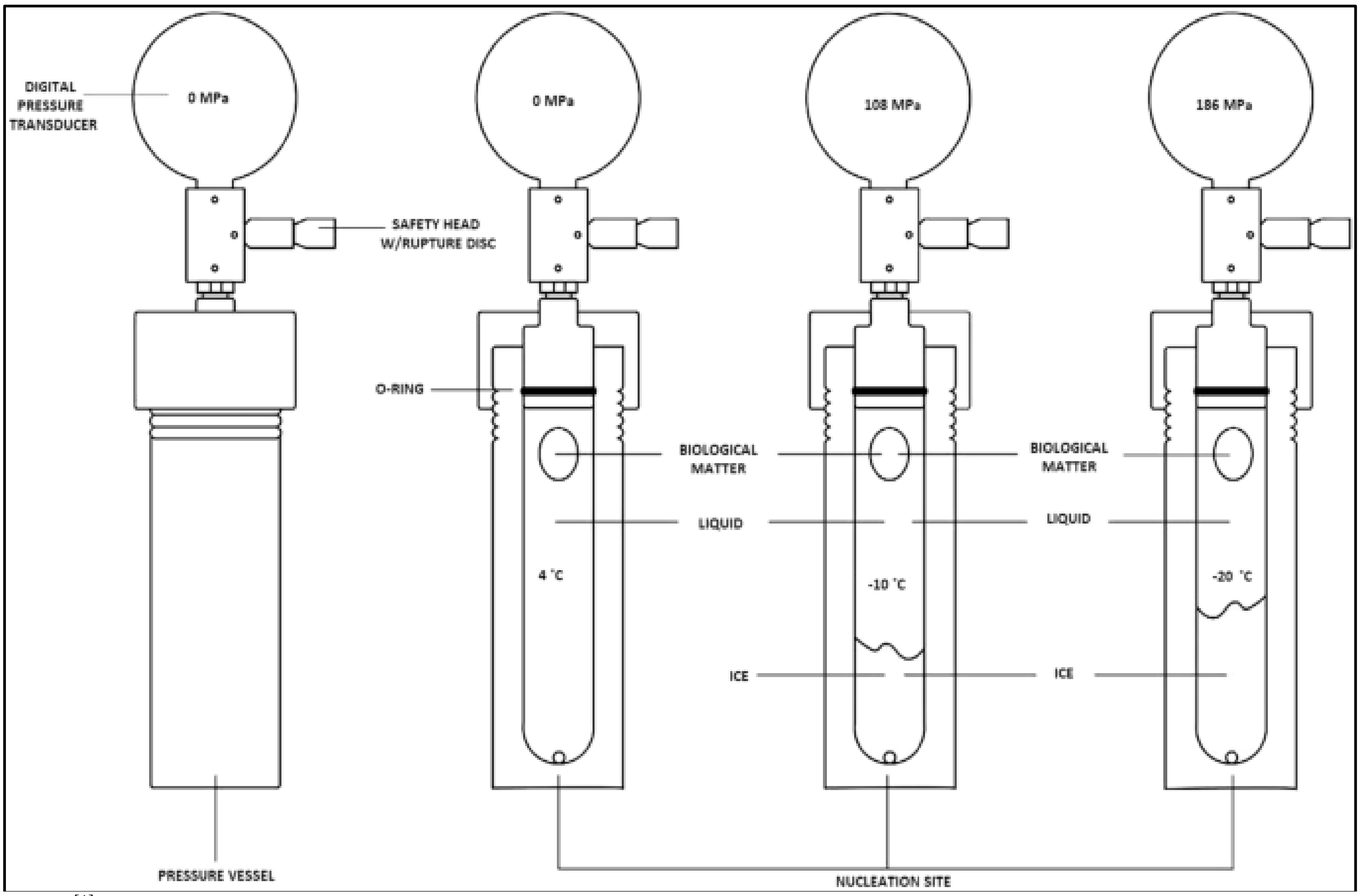


Fig 1: Phase diagram of pure water [7]

Isochoric Freezing and System Design

The first isochoric system was designed to withstand moderate pressures of up to 75.8 MPa and temperatures as low as -15 °C. Tools for measuring pressure and temperature were included with the system. Szobota's second study was theoretical in nature and focused on the vitrification of the aqueous phase in an isochoric system. A novel isochoric device that could withstand pressures up to 275 MPa and temperatures as low as 20°C was created by Preciado. The device made it possible to raise the experimental temperature and pressure readings in an isochoric aqueous phase system from -12 °C to -20 °C [8]. An isochoric system at 5 °C and 60 MPa was shown to support the survival of the worm *C. elegans* by the application of the proposed approach. The isochoric unit is a cylinder-shaped, double-walled, jacketed stainless steel cylinder composed of pressure transducers, carbon fiber composites, and powerful thermosets. The isochoric unit consists of a cylinder-shaped, double-walled, jacketed stainless steel cylinder with carbon fiber composites, powerful thermosets, and pressure transducers [9].

Dependent upon the system's temperature and pressure, rupture discs are employed in isochoric procedures. Regarding preservation, salt/sugar solutions also function similarly to hurdle technology. The area becomes a nucleation location for ice crystals that form after that. For food ingredients to stay in their aqueous phase and prevent the accumulation of ice crystals, a nucleator is required [2]. The equilibrium between the ice and solution within the chamber will persist if the external component remains constant. The chambers measure one inch in diameter inside, three inches in length, and two and a half inches on the outside. The material used to make them is stainless steel. The chamber is sealed and closed using a screw, a metal seal, and an additional electronic pressure transducer with a ruptured disc of 60 MPa. To regulate the temperature, the apparatus needs a water bath [10].



Source [1].

Fig 2: Isomochoric chamber and generalized freezing process schematic. Ice expansion will provide hydrostatic pressure, which lowers the freezing point of a system with restricted liquid volume with stiff walls and no air pockets. Until the system's freezing point and temperature are equal, the pressure will keep rising. Phases of liquid and solid will be in balance at this moment. Between 12% and 23% of the entire volume will be frozen at −4 and −7 degrees Celsius, in that order.

Food application of the isochoric process

1. Grape tomatoes

Tomatoes were used in an isochoric freezing experiment. Rich amounts of carotenes (especially lycopene), phenols, and vitamin C make tomatoes an important food source for humans. After harvesting, tomatoes—especially grape tomatoes—should be kept at 10 °C or higher to avoid chilling damage. Because of their extreme texture degradation, color change, and nutritional degradation during and after frozen storage, tomatoes are not appropriate for ordinary freezing. The isochoric system consists of a sealed OC-9 pressure chamber made of 316 stainless steel connected to an electronic pressure transducer. A 50:50 mixture of ethylene glycol and water was used to cool the equipment. The operating temperature of this system is −2.5 °C. Tomatoes preserved using isochoric preservation had a more uniform quality when compared to methods such as cold storage and IQF. Tomatoes with the best mass, morphology, color, and textural features. Moreover, the segregation of the cells during isochoric freezing preserved the ascorbic acid content, lycopene level, antioxidant properties, and phenolic components of tomatoes. Cryo-SEM demonstrates that isochoric freezing protects the nutritious value of tomatoes by preventing the formation of ice crystals and only marginally damaging tomato tissues [11].

2. Spinach

For the isochoric system, an alloy steel pressure chamber was provided by the High Pressure Equipment Company (Erie, PA, USA). The total volume's capacity was 66 milliliters. After being cooled in a recirculation bath, the electronic pressure transducer was attached to the pressure chamber. Three distinct spinach preservation techniques were compared to the effects of both industrially frozen and fresh spinach. In the initial technique, spinach leaves were submerged in an isochoric container containing 10° Brix sucrose concentration. There were numerous people in the room. The sucrose solutions at 10° Brix, and then firmly closed. It was then placed in a cooling bath with a recirculating temperature of -4.0 ± 0.9 °C. Within the chamber, the pressure increased to 29.7 ± 0.2 MPa. Leaf samples of spinach were analyzed after one to seven days. In the second approach, a plastic bag containing a 10° Brix sucrose solution was placed inside an isobaric system including spinach leaves. The bag was stored for a maximum of seven days at −4.0 ± 0.9 °C in a recirculating water bath. The third method involved vacuum-packing spinach leaves and keeping them in a recirculating bath at −4.0 ± 0.9 °C for a maximum of seven days. After every treatment, samples were allowed to thaw for 30 minutes at room temperature before being examined. Compared to isobaric freezing, isochoric freezing of spinach maintains more of the leaf's texture and qualitative qualities. Furthermore, samples kept for a week displayed better characteristics than commercially frozen isochoric samples. As there are no ice crystals in the spinach leaves when freezing, isochoric freezing has been linked to less cell damage [12].

3. Potatoes
Potatoes were preserved using straightforward isochoric freezing methods. To withstand the increasing pressures in the system with little distortion, a constant volume chamber is needed. For control, they require a pressure transducer. An O-ring constructed of 316 stainless steel, an inner capacity of 125 mL, a working pressure of 13 800 psi, and a test pressure of 20,000 psi characterize an OC-1 pressure vessel designed specifically for the isochoric chamber. My DAQ connector, an NI, and a pressure gauge are connected to the metal seal and screw-fastened constant volume cabinet. The experiment's data is recorded and shown using LabVIEW. For safety, a rupture disc limited the pressure to 60 MPa. A bath of water and ethylene glycol was used to cool the isochoric chamber [13].

4. Sweet cherries
Fresh cherries (6-7 g) were acquired from neighboring supermarkets and local growers and stored at 5 °C until being processed. The essential part of the isochoric system was the R1 pressure chamber, which was made of 4340 alloy steel. It had a combined volume capacity of 66 mL with measurements of 1 inch for internal width, 3.1/5 inches for external width, and 6 inches for internal length. The constant volume container was sealed with a metal seal and screw. Attached to the pressure chamber were a laptop and an electronic pressure transducer. The information was recorded and displayed using Additel's graphical software and data recorder. A mix of 50:50 ethylene glycol and water was used to cool the equipment. In order to preserve fresh cherries, three methods were used: individual rapid freezing (IQF), preservation for a whole day in an isochoric system at a temperature between -4 and -7 °C, and preservation for 24 hours at -4 or -7 °C in an isobaric system. When frozen at −4 °C, sweet cherries exhibited exact nutritional and quality attributes similar to those of fresh cherries. The cherry's texture was retained and drip loss was reduced at -4 °C thanks to isochoric freezing. As assessed, the absence of ice crystals in the delicious cherries during the freezing process was connected with the efficiency of isochoric freezing, which reduced cellular damage in cherry tissue, under cryo-SEM analysis. Despite being translucent from isochoric freezing, the cherries' hue was the closest to that of fresh cherry. Furthermore, because cell fragmentation was maintained throughout freezing, isochoric freezing at -4 °C preserved the antioxidant activity, phenolic compounds, and ascorbic acid of cherry [1].

5. Pomegranates
Pomegranates were washed in 200 liters of sodium hypochlorite solution to reduce the initial microbial burden. Pomegranate fruit husks have been physically processed in order to separate the arils. Four methods were used to store pomegranate fruits for one month: isochoric freezing at 2.5 °C and 12 MPa, cold storage at 5 °C and 95% relative humidity, isochoric super cooling at 2.5 °C and 0.1 MPa, and isobaric freezing at 2.5 °C and 0.1 MPa. The fruits were kept whole and the arils were freshly cut. When the entire pomegranate was isochoric supercooled, the color, antioxidant activity, texture, and ascorbic acid concentration all increased. For freshly cut arils, isochoric super chilling at −2.5 °C was one of the most successful preservation methods. Ascorbic acid concentration, microbial development, and color and texture preservation were all enhanced by isochoric super chilling. Visible microbiological deterioration and a decrease of quality in terms of volume, color, texture, and phytochemical content were the outcomes of cold storage. The majority of the mass loss was attributed to isobaric freezing, which also significantly altered the arils' color and texture. Compared to isochoric supercooling, isochoric freezing at 2.5 °C prevented the growth of microorganisms, but it adversely affected the pomegranate aril's color [14].

Conclusion
In conclusion, food loss remains a significant global issue, with approximately 1.3 billion tonnes lost annually. The rise in food consumption through 2050 further underscores the need for effective preservation techniques. Traditional freezing methods, while common, often compromise food quality due to ice crystal formation. Isochoric freezing presents a promising alternative by avoiding large ice crystals through high-pressure conditions, thus preserving the food's texture and nutritional value. Research has demonstrated the efficacy of isochoric freezing in maintaining the quality of various foods, including tomatoes, spinach, potatoes, cherries, and pomegranates. This method not only reduces food waste but also enhances the longevity and quality of frozen products, making it a valuable tool for future food preservation strategies.

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