**Isochoric freezing: An innovative technology for fruits and vegetables preservation**

**Charu Bisht1\* and Deeksha Semwal2**

**1&2 Research Scholar, Department of Food Science & Technology,**

**Govind Ballabh Pant University of Agriculture &Technology,**

**Pantnagar, U. S. Nagar, Uttarakhand, India**

**Corresponding Author e-mail:** charubisht3096@gmail.com**\***

**ABSTRACT**

Low-temperature preservation of food is a well-established technique. Despite the fact that traditional methods for food preservation are based on isobaric (constant pressure), it is often found that they produce a number of irrevocable modifications that considerably affect the characteristics of foods that have been frozen. The perishability of fruits and vegetables makes them susceptible to degradation. The shelf life of different fruits and vegetables must be extended while maintaining their physical and nutritional characteristics, thereby requiring a reliable preservation method. In recent years, there has been growing attention from both the research and commercial sectors towards isochoric freezing, which is proving to be a successful method for preserving food by maintaining a constant volume. Food is preserved longer during isochoric processing since it is carried out at a sub-zero temperature. This sustainability will reduce price fluctuations, solve issues of food safety and security, reduce post-harvest losses, and improve market viability. Additionally, poultry and other meat products with improved keeping qualities have proved the potentiality of this technique. The problems encountered during preservation can be solved by the technique of isochoric freezing. This freezing technique has the potential to substitute for every other method of preservation because it uses less energy to maintain the quality of food. It is an emerging area of food preservation that needs more attention from researchers as well as food manufacturers.

**Keywords-**Isochoric freezing, Isobaric freezing, Preservation

**I. INTRODUCTION**

Food products are preserved via isochoric freezing at below-freezing temperatures without the production of internal ice. A cooling system and a constant-volume chamber that can withstand the pressures that build up during processing make up the isochoric freezing system. In the enclosed chamber, the food product is heated to below-freezing temperatures while submerged in an isotonic solution. The phase diagram for the isotonic solution shows that freezing happens at a constant volume and that the temperature and pressure follow the liquidus curve. Ice forms in the solution during freezing and grows in volume, raising the pressure inside the sealed chamber. Le Chatelier's concept, however, results in a portion of the volume remaining unfrozen. The food is preserved at below-freezing temperatures with no internal ice formation when the chamber is made to contain the product in the non-frozen area. In traditional freezing methods, where freezing takes place at constant pressure (atmospheric pressures), this separation between ice and food is impossible. Under these circumstances, practically all of the food's water content will freeze once it hits the freezing point, making isochoric freezing a new way to prevent frozen foods' quality from degrading due to ice formation. Beyond its common household application, freezing is a traditional technique for food preservation, extensively utilised at various stages within the commercial food distribution network. The traditional method of freezing food entails bringing the food's temperature down to or below -18 °C. When food comes into contact with a freezing medium, it experiences a temperature drop because of heat transfer. Various factors, such as system configurations, the formation of ice crystals, phase changes, and freezing durations, affect the quality of food items. The freezing procedure presents several challenges, such as uneven freezing rates, increased costs, and the use of inappropriate temperature ranges or freezing conditions, which can have adverse consequences. Typically, food freezing involves five distinct stages. The preservation of biomaterials via isochoric pressure-aided supercooling, often known as "isochoric freezing," has lately received attention as a better method than traditional isobaric freezing along the entire food cold chain. The product's temperature is lowered to its initial freezing point (0 °C) during the pre-cooling phase. The super-cooling phase is brought on by further heat removal to temperatures below 10 °C. Following this phase of extreme cooling, ice starts to form, and then the latent heat of crystallisation is released. In the subsequent phase, the food's temperature continues to decrease as additional water transforms into ice and reaches the eutectic point. Eventually, the product's temperature aligns with that of the freezing medium. The intricate composition of the food matrix, which includes a mixture of solutes with different freezing points, adds complexity to the process. Nevertheless, during the freezing process, all foods go through these stages. The isobaric technique, which involves simultaneous changes in temperature and volume, is used in conventional freezing operations. The isobaric mechanism causes an unlimited amount of solution in the food to freeze. The formation of ice crystals within food can cause damage to the cellular structure of biological systems. Isochoric freezing offers a potential solution to mitigate the risk of cell integrity loss by overcoming these limitations.

**A. Food preservation methods**

Since the main methods of food preservation are all based on a very small number of variables, their applicability must therefore be constrained. The majority of the methods are focused on either slowing down or, in certain cases, completely preventing the growth of microorganisms. Responding to consumer preferences, some of the latest strategies involve more natural approaches, including modified packaging, the application of protective cultures, the bacteriocin utilization, and other microbial products, as well as enzymes. In contrast to inhibitory approaches, few of the most popular techniques work primarily by inactivating the target microorganisms; in fact, heating is the only technique that is primarily utilised for this goal. The majority of the newer or emerging techniques, however, do act by direct inactivation. Examples include: (a) irradiation; (b) the application of high hydrostatic pressure; (c) high-voltage electric discharge (electroporation); (d) ultrasonication combined with increased temperature and slightly raised pressure (manothermosonication); and (e) the addition of bacteriolytic enzymes (lysozyme).

**Table 1**.Existing and emerging antimicrobial techniques employed to preserve foods and to achieve desired shelf life

|  |  |  |
| --- | --- | --- |
| **Objective** | **Preservation factor** | **Method of achievement** |
| Reduction or inhibition of growth  Inactivation of microorganisms | Low temperature  Low water activity  Restriction of nutrient  availability  Lowered oxygen  Raised carbon dioxide Acidification  Alcoholic fermentation  Use of preservatives | Chill and frozen storage  Drying, curing and conserving Compartmentalization in water-in-oil  emulsions  vacuum and nitrogen packaging  Modified atmosphere packaging  Addition of acids: fermentation  Brewing, fortification  Addition of preservatives: inorganic (sulphite, nitrite); organic (propionate, sorbate. benzoate. parabens); antibiotic (nisin, natamycin) |
| Heating  Irradiating  Pressurizing  Electroporating  Manothermosonication  Cell lysis | Pasteurization and sterilization  Ionizing irradiation  Application of high hydrostatic pressure  High voltage electric discharge  Heating with ultrasonication at slightly raised pressure  Addition of bacteriolytic enzymes (lysozyme) |

**(Source: Gould (1989)**

The principal preservation strategies currently used to prevent or delay spoiling are temperature reduction, pH reduction, water activity decrease, and the use of heat. However, these and other strategies are increasingly being utilised in conjunction with combination preservation or hurdle technologies, and it is widely anticipated that these approaches will see increased implementation in the future. While many of the most commonly used combination strategies were developed empirically.

1. **Freezing at constant volume**

Almost all biological materials have substantial water content, and lowering the temperature to the point where water freezes can lead to several types of damage. The quality of the material that is stored as a whole differs noticeably between intracellular and extracellular ice production. In the food industry, research is underway to explore the application of isochoric freezing as a means to prolong the shelf life of perishable products, diminish food waste, and preserve nutritional and sensory attributes. The biological materials were kept in various isochoric environments for observation of the alterations that take place during processing. This section is centered on the utilisation of isochoric freezing as a method for preserving food.

1. **Principle of isochoric freezing**

The isochoric unit consists of a cylindrical, double-walled, stainless steel chamber equipped with carbon fibre composites and robust thermo-set materials. Pressure transducers are integrated into this chamber. To facilitate isochoric operations within the system, rupture discs are employed based on pressure and temperature conditions. Sugar or salt solutions are also utilised for preservation purposes, operating similarly to hurdle technology. Ice crystals introduced into these solutions act as nucleation sites. This nucleation is essential to maintain food ingredients in their aqueous phase without allowing the formation of ice crystals.

The chamber's design is such that it maintains equilibrium between ice and the solution as long as external factors remain constant. The chamber is constructed using stainless steel and has specific dimensions. It is sealed securely with a screw and metal seal and is equipped with an omega electronic pressure transducer with a rupture disk rated at 60 MPa. Additionally, a water bath is employed in the system to regulate temperature.

**Table 1:** Comparison between isochoric and isobaric freezing

|  |  |  |
| --- | --- | --- |
| **Parameters** | **Isochoric freezing** | **Isobaric freezing** |
| Constant parameter | Volume | Pressure |
| Pressure | High pressure (around 30MPa or above) | Low pressure |
| Temperature | Varies (-4 to -15 0C) | Varies (-18 0C or below) |
| Ice formation | Exterior of the food | Within the food |
| Energy consumption | Low (as only some portion is frozen) | High |
| Nutritional quality | Less affected | More affected |

1. **Isochoric freezing of fruits**

People are aware of the beneficial effects of consuming fruits. Fruits are essential components of a balanced diet due to the presence of vitamins, mineral salts, and dietary fibres that have health-improving or disease-preventing characteristics. Fruits and vegetables are perishable items that rapidly deteriorate, so it is essential that they remain stable after harvest and during subsequent storage. An efficient preservation strategy is needed to increase the shelf life and retain the physical and nutritional qualities of seasonal fruits. Due to its perishability, and in order to minimize the waste, a proficient preservative technique is required. Technology used to preserve food typically slows the reactions that cause quality degradation and prevents the growth of microorganisms. Among such techniques, freezing is a successful and reliable method.

The significant factors responsible for the quality and stability of frozen fruits and vegetables are the product itself, the method of freezing, and the packaging. PPP factors is an acronym used for these three influential factors. The qualities of the finished product are greatly influenced by the type of processing employed. The process of freezing allows for the regulated removal of heat from the product at a steady uniform rate until the heat still present is equivalent to its equilibrium following stabilization. Consequently, the production of ice crystals results in disruption of cellular structure. Once the cell membrane loses its permeability, ice crystals start to form in the extracellular space and move towards the cytoplasm. Cells decompartmentalize as an outcome of the formation of ice crystals, which prevent the water from returning to the intracellular medium while thawing. As a result, the cells become less turgid, and their texture may significantly deteriorate. These changes might also encourage drip loss during thawing. Fruit tissues are more sensitive and additionally more prone to freezing, which can have devastating effects on cell turgidity and firmness. Conventional freezing techniques use an isobaric method where temperature and volume change simultaneously. The isobaric mechanism causes an unlimited amount of solution in the food to freeze. Conventional freezing techniques use an isobaric method where temperature and volume change simultaneously. The isobaric mechanism causes an unlimited amount of solution in the food to freeze. Isochoric freezing of biological systems can circumvent these restrictions and reduce the possibility of deterioration of cell structure. Food products are preserved via isochoric freezing at below-freezing temperatures without the production of ice inside the products. The phase diagram for the isotonic solution reveals that freezing takes place at a constant volume and that the temperature and pressure follow the liquidus curve. The food is preserved at below-freezing temperatures with no intracellular ice formation as the chamber has been created to keep the product in the non-frozen region. In traditional freezing methods, where freezing proceeds at constant pressure (atmospheric pressures), it is difficult to separate ice and food (Bilbao-Sainz *et al.,* 2021). Shifting to isochoric freezing results in minimal alterations in fruit quality.

1. **Effect on nutritional characteristics of fruits**
2. **Ascorbic acid content**

Ascorbic acid is primarily obtained from fruit juices. The essential antioxidant ascorbic acid, generally known as vitamin C, is widely present in plants. Fruits are a rich source of ascorbic acid and are the primary source of human intake of ascorbic acid. Ascorbic acid has an impact on fruit ripening, influences fruit's ability to withstand stress, and regulates fruit growth and postharvest storage. Ascorbic acid (AA) is likely to deteriorate when subjected to oxygen, a pH shift, an increase in temperature, or pressure, as it is quite sensitive to these factors. Food manufacturers’ primary objective is the preservation of AA in fruit and vegetable products during processing.

The study examined the viability of isochoric freezing for the preservation of tomatoes. Due to their significant texture degradation, color change, and nutritional loss during the freezing process and subsequent frozen storage, tomatoes are not appropriate for traditional freezing. Fresh grape tomatoes were found to have 196 + 21 mg/100 g d.b. of ascorbic acid. Under isochoric preservation, tomatoes preserved their ascorbic acid content for four weeks. The sample lost 17% of its ascorbic acid concentration after two weeks, but no more losses occurred after this. While just 10% of the ascorbic acid in the isobaric sample was retained at the end of storage, it had the lowest ascorbic acid level (Bilbao- Sainz *et al.,* 2021).

Fresh Rainier cherries had a total ascorbic acid content of 5.3 + 0.8 mg/100 g. Ascorbic acid content was retained in cherries that were kept under isochoric conditions (Bilbao-Sainz *et al.,* 2019). Also, the ascorbic acid concentration of the samples was unaffected by a pressure increase from 30 MPa to 62 MPa. One of the investigations demonstrated that hydrostatic pressure had no effect on covalent bonds and did not alter low molecular weight food compounds like vitamins. Indeed, Patras, Brunton, Da Pieve, and Butler (2009) concluded that ascorbic acid concentrations in strawberry and blackberry purées did not significantly change when pressures up to 600 MPa were applied. In contrast, considerable ascorbic acid losses were caused by IQF and isobaric freezing. This includes 72% loss following the IQF procedure, 63% loss at 4 °C under isobaric freezing, and 51% loss at 7 °C under isobaric freezing. A 12% drop in ascorbic acid content as soon as cherries were frozen at -18 °C. Ascorbic acid oxidase-mediated enzymatic oxidation in the presence of oxygen is most likely the reason behind the degradation of ascorbic acid. Since enzymatic reactions still happen when non-frozen water is present, they are slowed down but not entirely abolished in frozen goods. Isobaric and IQ freezing elevated these enzymatic activities in contrast to isochoric freezing. As the freezing inside the cherry damaged the cell membranes, which favour enzyme substrate-interactions while thawing, the isobaric and IQ freezing elevated these enzymatic activities in contrast to isochoric freezing.

In this study, the impact of isochoric supercooling at -2.5 °C and isochoric freezing at -2.5 °C/12 MPa on the quality characteristics of whole pomegranate arils (cv. "Wonderful") and fresh-cut arils kept for 30 days was examined and compared with cold storage at 5 °C/95% RH and isobaric freezing at 2.5 °C/0.1 MPa. Fresh pomegranate arils had an ascorbic acid concentration of 12.68 mg per 100g of juice. Following isochoric supercooled samples and isobaric frozen samples, the isochoric frozen samples showed the greatest increase in AA concentration as a result of pressure-induced impregnation. The movement of AA to the arils through cell membranes when they break down during storage may account for the increase in AA concentration for isochoric supercooled samples. Ice crystals may have harmed the integrity of the cellular compartments in isobaric frozen arils. Mass transfer between the arils and the surrounding 16% sucrose/0.5% ascorbic acid solution occurred as a result of the cellular membranes losing their semi-permeability (Bilbao-Sainz *et al.,* 2022).

1. **Antioxidant activity**

Antioxidant activity represents the ability of a bioactive compound to preserve cell structure and function by efficiently removing free radicals, suppressing lipid peroxidation events, and avoiding other oxidative damage. It serves as the basis for numerous other biological processes, including those that fight cancer, inflammation, and ageing. Furthermore, antioxidant activity has been linked to the prevention of chronic diseases, including cancer, diabetes, and cardiovascular disease. Therefore, it is crucial for human health to conduct in-depth research on natural antioxidants, such as those found in fruits and vegetables.

According to studies, antioxidant substances can improve human health by scavenging free radicals and preventing the oxidative processes that cause degenerative diseases. The anthocyanins, flavonoids, and total phenolic content of cherries are primarily responsible for the fruit's high antioxidant activity. Lower than the previously reported values of 3.3 + 0.1 mg TE.g-1 and 6.8 + 0.4 mg TE.g-1 in cherry flesh and peel, respectively. The antioxidant activity of fresh cherries was found to be 1.9 + 0.1 mg TE.g-1. Antioxidant activity did not change much when frozen at -4°C in isochoric conditions. But cherries stored under isochoric conditions at -7°C exhibited a seemingly higher antioxidant activity. Cherries that were frozen under isobaric conditions showed a small decrease in antioxidant activity. (Bilbao-Sainz et al., 2019).

Fresh tomatoes were shown to have an antioxidant activity of 16.6 + 2.3 mg TE/g d.b. Soluble antioxidants, primarily ascorbic acid and soluble phenolics contributed 92% of tomatoes' total antioxidant activity, while lipophilic antioxidants, primarily lycopene and lipophilic phenolics, contributed only 8% out of total. Antioxidant activity did not significantly change after freezing at -2.5 °C in isochoric conditions. The degradation of bioactive chemicals caused a progressive decline in antioxidant activity over time in the samples preserved by the other methods (Bilbao-Sainz *et al.,* 2021).

Pomegranate fruits' antioxidant activity was predominantly due to hydrolysable tannins (Punicalagins and Punicalins) and phenolic acids (such as ellagic acid). The antioxidant activity in fresh pomegranate arils was determined to be 3.96 ± 0.37 mg TEg−1 from the DPPH assay and 3.94 ± 0.34 mg TEg−1, from the ABTS•+ assay. During cold storage, the antioxidant activity of the arils from whole pomegranates decreased. Regardless of the postharvest preservation method, the antioxidant activity of fresh-cut arils was lower than that of whole pomegranate arils. The highest antioxidant activity was found in the isochoric supercooled and cold stored arils, while the lowest antioxidant activity was observed in the isochoric frozen and isobaric frozen arils (Bilbao-Sainz *et al.,* 2022).

1. **Effect on mechanical properties of fruits**

The mechanical characteristics of fruits and vegetables influence the texture of food products. In order to select suitable mechanical processing methods, the best time to harvest, and detachment techniques, an investigation of their food mechanical properties can be extremely beneficial. In the collection, processing, and storage of fruits and vegetables, mechanical properties can be used to anticipate probable harm. All textural qualities have been affected as a result of cold storage, isochoric freezing, and isobaric freezing. Isochoric frozen pomegranate arils experienced the most texture degradation. These samples lost 22% of their hardness, 41% of their crispiness, and 37% of their crunchiness, showing pressure-related physiological problems. Similarly, due to continuous metabolic processes, cold-stored arils lost 22% of their hardness, 29% of their crispiness, and 30% of their crunchiness. Substantial deviations show that partial freezing of isobaric frozen arils resulted in significant changes in textural qualities. Isobaric frozen samples lost 15% hardness, 7% crispiness, and 17% crunchiness on average. The softening and loss of crunchiness could have been caused by the aril's immersion in the aqueous solution (Bilbao-Sainz *et al.,* 2022). The integrity of the cell membranes and walls was weakened by the development of ice during isobaric freezing, which led to the most texture loss. This resulted in cell lysis and subsequent water and cellular component leakage, softening the arils and reducing the crunchiness and crispiness.

When buying a product for the first time, consumers make decisions on the basis of appearance, but subsequent purchases are generally influenced by quality elements like texture. In order to increase consumer appeal, frozen cherries should have a feel that is similar to fresh cherries. The best mechanical qualities were found in cherries that had been isochoric preserved. When compared to fresh cherries, cherries stored at -4 °C under isochoric conditions revealed no noticeable variations in the maximum force, elasticity modulus, or strain fracture values. High hydrostatic pressures can damage cell structures and result in a loss in cell membrane permeability, due to which lowering the temperature from -4 °C to -7 °C induces a slight decrease in sample firmness and rigidity. Comparatively, IQ freezing and isobaric freezing led to the biggest changes in textural characteristics. The firmness value of the cherries was much lower than that of fresh cherries, demonstrating that freezing affected the strength of the cell walls and membranes. For these samples, the elastic modulus also experienced a considerable value loss, indicating a more elastic behaviour coupled with a reduction in turgidity. Because the cherries were not hard enough to rupture or break, the samples did not fracture during the compression testing. There were no statistically significant changes between samples frozen using IQF or under isobaric conditions in terms of firmness or modulus. As a result of cell lysis and subsequent water and cellular component loss, both slow and quick-freezing rates resulted in significant sample softening. According to Kong et al. (2017), thawed cherries lost all of their firmness to the touch and developed a very mushy texture after being kept at -20 °C for 31 days. Additionally, Alonso, Rodriguez, and Canet (1995) discovered that the hardness and elasticity modulus values of IQF cherries decreased. Furthermore, Alonso, Tortosa, Canet, and Rodriguez (2005) discovered that intracellular pectin leakage, which led to the destruction of calcium bridges and the loosening of cell tissue, was a significant factor in the loss of firmness. Since the texture was similar to that of fresh Rainier cherries, the results suggested that preservation under isochoric settings was better than IQ freezing and preservation under isobaric temperatures (Bilbao-Sainz *et al.,* 2019).

1. **Effect on visual and colour characteristics of fruits**

Along with flavor and taste composition, the fruit's color is one of the most significant biochemical traits for grapes consumers. Minor color difference values (E\*) were obtained from arils that were stored as whole fruits using isochoric supercooling or isochoric freezing because all color parameters were maintained. While there was evident variation during cold storage, as cold storage greatly lowered color tone (hue angle, h°), and isobaric freezing drastically reduced h° and yellowness (b\*) attributes. The redness (a\*) values of freshly cut arils were not considerably altered by the various postharvest procedures. However, b\* and h° levels were substantially lowered by all postharvest treatments. The anthocyanin pigment is responsible for the color of pomegranate arils. For samples kept in the refrigerator, the decrease in their visual appeal was noticeable. Refrigerated samples exhibited microbial degradation with the growth of external surface mycelia and mild browning spurred on by the oxidation of phenolic chemicals. During isochoric supercooling, the distinctively vivid color of fresh arils was preserved, although the color tone appeared to have faded. In contrast, after isochoric or isobaric freezing, the distinctive vivid color and color tone of pomegranate arils vanished. Due to the movement of pigments from pulp to solution, enzymatic browning, and degradation of anthocyanins, damaged cells may have rapid discoloration (Bilbao-Sainz *et al.,* 2022).

Due to the tendency of grape tomatoes to lose weight during storage, made the tomatoes shriveled and dehydrated after three weeks. Almost all of the tomatoes that had been kept at 10 °C were of inferior quality. According to Cantwell, Nie, and Hong's (2009) research, grape tomatoes kept their visual appeal for 12 days when kept at 10 °C. IQF and isobaric frozen tomatoes had the least appeal in terms of appearance. The peel of these tomatoes was separated from the tomato pulp and appeared mushy. Compared to IQF, isobaric freezing had adverse impacts on tomato appearance. Isochoric freezing and cold storage barely changed the L\* a\* b\* values for colour. The low values of ΔE\* suggested that these tomatoes' colour was consistent with the fresh sample during the whole preservation period. When compared to fresh samples, IQF and isobaric samples were more yellow (had higher b\* values). For IQF samples, this behaviour was more pronounced. Additionally, the isobaric and IQF samples had greater ΔE\* values than the cold-stored and isochoric frozen tomato samples. Tomatoes and tomato products have been known to change colour when frozen. The usual colour change during storage is characterised by an increase in the yellow character and concurrent lightening of the colour (Lisiewska and Kmiecik, 2000). The carotenoid concentration of frozen tomatoes decreased due to enzymatic oxidation. IQF samples had a higher b\* value in comparison to isobaric samples.  This might be because of greater carotenoid oxidation due to air circulation during frozen storage at −20 °C in IQF samples (Bilbao-Sainz *et al.,* 2021).

Isochoric frozen cherries showed a darker color and a slight increase in a\* (redness) and b\* (yellowness) values. This most likely occurred as a result of the sucrose solution filling the pores of the tissues of cherry tissue, offering the fruit a translucent appearance. Indicating that hydrostatic pressure over the experimental range had no effect on the degradation of β-carotene, the main chemical responsible for the yellow colour of Rainier cherries, or phenolic compounds, samples at -4 °C and -7 °C displayed no apparent differences in colour. In contrast, when compared to fresh samples, lightness (L\*) and b\* values for isobaric and IQ frozen samples considerably reduced, although a\* values increased. When the temperature dropped below freezing, this behaviour was more pronounced. The enzymatic oxidation of native enzymes like lipoxygenase is likely what led to the breakdown of β-carotene. Additionally, polyphenol oxidases and peroxidases may have caused enzymatic browning. Damage to the membrane most likely enabled these degradation reactions to accelerate. The variations in colour of the cherries were most likely caused by interactions between enzymes and substrates that were aided by membrane degradation during freezing. The lowest chromatic difference in cherries was caused by isochoric preservation at -4 °C and -7 °C, whereas the highest chromatic difference was caused by IQ freezing (32%). The samples frozen at -4 °C and -7 °C in an isobaric system had chromatic differences of 17% and 30%, respectively.

Anthocyanins and flavonoids are sensitive to temperature and other environmental conditions, which is responsible for the black color of the grapes. The production of ice crystals during traditional freezing can harm grape cells and cell walls, releasing colours and changing the colour of the grapes. Black grapes must be frozen without maintaining constant pressure in order to be preserved under isochoric conditions (constant volume). The quality of black grapes can be maintained by isochoric preservation, which can reduce ice crystal formation and damage to the grape cells and cell walls. By decreasing the loss of grape juice, soluble sugars, and pigments, respectively, isochoric preservation can also aid in maintaining the Brix level and colour of black grapes (Campean *et al.,* 2023).

1. **Effect on structural properties of fruits**

Cryo-SEM pictures of the tissue structure at the cellular level could be beneficial for understanding the impact of freezing on cherry structure. Fresh cherry cells have a turgid, compact appearance. Air occupied some intercellular gaps. Cells in cherries that were isochorically frozen at -4°C kept their walls and membranes intact. The external sugar solution appeared to fill intercellular gaps, but the cherry's structural integrity was unaffected. Under isobaric conditions, freezing at -4°C or -7°C caused significant alterations in the structure of cells. Due to cellular component mobility in these samples, fluid filled the intercellular gaps. Additionally, due to the lack of intracellular pectin, some cells seemed loose with wide intercellular spaces. Dehydration also resulted in the cell walls folding and buckling. The tissue suffered severe damage as a result of IQ freezing. Some of the cell walls in this sample could be distinguished as having irregular forms because they had a lighter appearance. However, due to the release of intracellular content, the tissue seemed homogenous, and it was challenging to distinguish between intracellular and extracellular volumes because both were loaded with water and particulates (Bilbao-Sainz *et al.,* 2019).

After 4 weeks of preservation, cryo-SEM pictures were taken to show how the various preservation methods affected the tomato cells. A thin layer of epidermal cells and numerous layers of relatively small, flattened cells make up the tomato "skin" or peel. Polyhedral cells of different sizes and shapes form parenchyma. They resemble skin cells in size and are divided by numerous intercellular gaps. Due to the lack of ice crystal formation and low processing pressures, the tissue of isochoric preserved tomatoes resembled that of the fresh sample. Despite the intercellular gaps seeming to be fluid-filled, the cells were unharmed. The corrugation and folding of the cells, as well as the collapse of nearly all intercellular gaps, could be revealed by the micrographs. The cell walls in these samples may have buckled and folded as a result of water loss brought on by senescence processes and storage. In parenchyma tissue from IQF samples, some cells seemed to have entirely lost their cellular structure, while other cells appeared to have retained it. Fast freezing rates resulted in a huge number of tiny ice crystals, which may have assisted in keeping some cell compartments frozen. In contrast, the tomatoes maintained under isobaric conditions experienced total histological destruction. Large ice crystals are produced by slow freezing, which severely damages the cells. This was most likely caused by an osmotic imbalance brought on by ice crystal formation as well as mechanical damage. (Bilbao-Sainz *et al.,* 2021)**.**

1. **Isochoric freezing of vegetables**

Finding effective preservation techniques that increase the shelf-life of vegetables while keeping their fresh-like qualities and nutritional value is one of the issues faced by food producers. Vegetables can be preserved easily and effectively by freezing them, which extends their shelf life. Traditional freezing, on the other hand, disturbs cell integrity and compartmentation, resulting in the death of the cells. This intensifies unfavorable physical, chemical, and metabolic processes that result in nutrient loss, texture alterations, and color changes. In order to meet industrial needs in an economical way, frozen storage results in a gradual, cumulative and permanent loss of fresh-like qualities and overall quality. Food products stored in isochoric systems will experience fewer temperature fluctuations during transport and storage because under isochoric conditions, temperature fluctuations result in phase changes rather than sensible temperature changes. This implies that the food industry can benefit from isochoric freezing capabilities without committing massive infrastructure changes to the current refrigeration infrastructure.

1. **Effect on nutritional composition of vegetables**

Spinach has high antioxidant activity because of the comparatively high concentrations of ascorbic acid, chlorophyll, and total soluble phenols it contains. Although the amount of loss varied depending on the freezing technique, all samples showed a reduction in ascorbic acid content, chlorophyll content, total soluble phenolic content, and antioxidant activity. In comparison to the isobaric samples, the isochoric samples generally had a higher nutritional content. The isobaric sample submerged in solution also contained more nutrients than the isobaric sample vacuum-packed. The spinach that was sold frozen has the fewest nutrients.

Fresh spinach was found to contain 31.1 - 2.9 mg/100 g (w.b.) of ascorbic acid, which is comparable to 47.4 -9.6 mg g-1.After a day, the isochoric sample still contained 32% ascorbic acid, but only 10% after seven days.

In contrast, the isobaric sample immersed in a solution retained 15% of its ascorbic acid content after 1 day, with no additional losses observed after 7 days. However, the isobaric vacuum-packed sample exhibited a further 10% reduction in ascorbic acid compared to the isobaric sample immersed in the solution. Notably, the commercially frozen sample displayed the lowest level of ascorbic acid content, measuring at 1.43 + 0.10 mg g-1. The antioxidant activity of fresh spinach leaves was determined to be 2.02 mg TE/100g (w.b.), equivalent to 337.0 - 7.3 mg TE/g. All frozen samples exhibited a decline in antioxidant activity over time. Specifically, the isochoric sample displayed the smallest decrease in antioxidant activity, showing a reduction of 54% after 1 day and 75% after 7 days. In contrast, the isobaric sample immersed in the solution experienced a 77% decrease in antioxidant activity after only 1 day, with no further declines noted after 7 days. Notably, the isobaric vacuum-packed sample demonstrated reductions of 82% and 88% after one and 7 days, respectively. The commercially frozen spinach exhibited the lowest antioxidant activity at 23.1 - 5.5 mg TE/g (Bilbao-Sainz *et al.,* 2020).

In one of the studies, potato cylinders were subjected to isochoric freezing, and a comprehensive exploration was conducted using a full factorial approach involving three different processing methods (immersion in water, vacuum packaging, and immersion in an ascorbic acid solution), four varied freezing temperatures and pressures (-3 ̊C/37 MPa, -6 ̊C/71 MPa, -9 ̊C/101 MPa, and -15 ◦C/156 MPa), and two distinct average compression rates (below 0.02 and above 0.16 MPa/s). The study aimed to investigate how these process variables influenced the critical quality characteristics of frozen potatoes upon thawing. The observed mass changes ranged from a 14% increase to an 11% decrease, depending on the specific isochoric freezing conditions employed. Notably, samples immersed in water and frozen at temperatures of -3 °C, -6 °C, and -9 °C displayed increases in mass, with mass gain decreasing from 13.6% at -3 °C to 9.2% at -9 °C. Conversely, at the lowest freezing temperature of -15 ̊C, the potato samples lost 10.9% of their mass. The mass increase was attributed to water absorption, driven by differences in osmotic potential between the potato cells and their surrounding environment. The role of pressure was complex, as vacuum-packed samples experienced greater mass loss at -15 °C (10.3%) compared to -3 °C (3.4%), indicating an escalation in cell damage at higher pressures. Furthermore, potato samples immersed in the ascorbic acid solution showed mass gain at the highest freezing temperature of -3 °C. In terms of volume changes, these ranged from a 30% increase to a 14% decrease. The most substantial volume increase occurred at the highest temperature (-3 °C) due to increased turgor pressure within the cells and swelling of cellular components as water infiltrated the tissue. However, at lower freezing temperatures, the increase in volume due to swelling was counteracted by a decrease resulting from greater cell damage at higher pressures. Thus, the overall volume changes depended on the interplay between these two factors. Vacuum-packed potato samples experienced more significant volume losses at lower temperatures due to increased cell lysis from higher pressure conditions. In contrast, potato samples immersed in the ascorbic acid solution generally did not exhibit significant volume changes across most experimental conditions. Only a 5% volume increase was observed at -3 ◦C and 0.16 MPa/s due to pressure-induced impregnation, while an 8.7% volume decrease occurred at -15 ◦C and 0.16 MPa/s due to cellular damage. These findings underscored the notion that lower compression rates resulted in less cellular damage for samples processed in an isotonic solution (Zhao *et al.,* 2021).

The growing demand for more convenient food options has recently driven the expansion of minimally processed potato products. This study delved into the impact of isochoric freezing on pre-peeled potato cubes, comparing it with isobaric freezing and individual quick freezing (IQF) followed by frozen storage at -20 °C for 4 weeks (Bilbao- Sainz *et al.,* 2020). The results showed that isochoric freezing (at -3 °C/30 MPa) yielded superior outcomes in terms of lower drip loss, reduced volume shrinkage, as well as better-preserved texture and microstructure compared to the other freezing methods. Initially, fresh potato tubers had a Total Soluble Phenolics (TSP) value of 0.30 ± 0.01 mg GAE/g wet basis (w.b.). During isochoric freezing, there was an 81% increase in TSP values after 7 days, with no significant changes observed at longer freezing times. This increase in phenolic content during isochoric freezing might be attributed to heightened phenylalanine ammonia-lyase activity resulting from cell injury, which in turn led to an increase in the concentration of phenolic compounds. TSP contents also significantly increased in tubers subjected to isobaric freezing and IQF treatment. For isobaric freezing, TSP increased by 12% after 7 days and 45% after 4 weeks, while IQF-treated tubers displayed a continuous rise in TSP content, increasing by 37% after 7 days and 82% after 4 weeks. In the case of isobaric and IQF samples, the change in TSP content seemed to be influenced by two opposing factors: an increase caused by cell injury and a decrease due to the release of PPO enzymes interacting with phenolic compounds. The Antioxidant Capacity (AOX) in fresh potatoes was measured to be 1.6 ± 0.3 mg TE/g w.b. Potato cubes prepared using all freezing methods exhibited similar AOX trends as the phenolic contents, suggesting that the increase in AOX could be attributed to the rise in phenolic content during storage. Regarding Ascorbic Acid (AA) content, fresh potatoes were found to contain 10.9 ± 0.3 mg/100g (w.b.). However, in all freezing treatments, there were decreases in AA content over time. After 4 weeks, the isobaric sample retained the highest AA content at 10.4%, followed by the isochoric sample at 6.9%, and the IQF sample at 1.2%. The decline in AA contents could be attributed to the release of ascorbate oxidase from damaged cells during the peeling, cutting, and freezing processes, leading to increased interactions between ascorbate oxidase and AA.

1. **Effect on mechanical properties of vegetables**

The fresh potato had maximum stress and elasticity modulus values of 0.61 ± 0.04 N/mm² and 2.8 ± 0.1 MPa, respectively (Bilbao-Sainz et al., 2020). When it came to isochoric frozen potato cubes, there was a notable increase in maximum stress after 2 weeks. According to Basak and Ramaswamy (1998), this enhancement in texture in response to high-pressure processing is likely due to the activity of pectinmethylesterase and the increased densification of the cellular structure, resulting from the removal of air from the tissue. However, the elastic modulus of isochoric frozen potatoes declined by 39% after 3 weeks. In contrast, isobaric and IQF-treated potatoes did not fracture during the compression tests because they had lost their rigidity. These samples exhibited a significant reduction in both maximum stress and elastic modulus after just 1 week. In particular, the maximum stress of thawed potatoes decreased by roughly 80% and 68% under isobaric and IQF conditions, respectively. Both freezing methods showed no substantial changes in maximum stress after 1 week. Similarly, the isobaric and IQF samples displayed a similar pattern of nearly 98% reduction in elasticity modulus values after 1 week, with no further alterations beyond that point. The formation of ice crystals during freezing under atmospheric pressure resulted in cell damage, causing the tissues to lose their turgor pressure and stiffness, ultimately leading to their softening (Zdunek, Gancarz, Cybulska, Ranachowski, and Zgórska, 2008).

The study aimed to assess the preservation of baby-leaf spinach through isochoric freezing. Among the various freezing methods tested, the isochoric sample exhibited the most favorable mechanical properties. These samples experienced a slight reduction in rigidity over time but maintained their crispiness, as evidenced by their short distance at break and peak force values, which were similar to those of fresh spinach. In contrast, the isobaric sample that was immersed in a solution displayed a notable decrease in elastic modulus, indicating a more elastic texture and a loss of crispiness. In fact, these samples had elasticity modulus values comparable to those of commercially frozen spinach. The isobaric sample also exhibited peak force and distance to break values higher than those of fresh samples, although these differences were not statistically significant. This can be attributed to the greater flexibility of the spinach leaves. Interestingly, the mechanical properties of the isobaric sample immersed in the solution and the isobaric vacuum-packed sample did not significantly differ. Moreover, these samples showed no significant variations in mechanical properties after 1 and 7 days of frozen storage (Bilbao-Sainz *et al.,* 2020).

1. **Color characteristics of vegetables**

Color in fruits and vegetables is a result of natural pigments, many of which undergo changes as the plant matures and ripens. The key pigments responsible for color include fat-soluble chlorophylls (green) and carotenoids (yellow, orange, and red), as well as water-soluble anthocyanins (red, blue), flavonoids (yellow), and betalains (red). In this research, the effectiveness of isochoric freezing in preserving the quality of baby-leaf spinach was examined. The initial color parameters for fresh baby-leaf spinach were measured as follows: lightness (L\*) at 43.9, greenness (a\*) at 8.0 and yellowness (b\*) at 20.7. Statistical analysis of color changes in frozen spinach leaves revealed significant differences in the L\* parameter value, indicating a darker color compared to fresh spinach. The greenness (a\*) values remained relatively stable across all spinach samples, while the b\* values for isochoric and isobaric samples immersed in a solution decreased significantly after 7 days of freezing. Additionally, the spinach leaves appeared somewhat translucent. The isobaric and commercially frozen samples exhibited greater translucency compared to the isochoric sample (Bilbao-Sainz *et al.,* 2020).

In a separate study involving potato cylinders frozen within an isochoric system, various processing methods, freezing conditions, and compression rates were explored. The appearance of fresh potatoes and isochoric frozen potatoes after thawing at -15 ◦C for one hour was observed. Samples packed in an ascorbic acid (AA) solution retained their yellow color regardless of freezing temperature, pressure, or compression rate, whereas vacuum-packed samples and those directly immersed in water exhibited some browning. Additionally, higher compression rates were associated with increased browning. Notably, there was a significant interaction between compression rate and processing procedure, indicating that, regardless of the compression rate, samples immersed in AA solution maintained their color, while vacuum-packed samples exhibited higher browning index values compared to those immersed in water. Furthermore, samples immersed in water had notably lower browning index values at the lower compression rate (Zhao *et al.,* 2021).

1. **Impact of isochoric freezing on macromolecules**

Research on proteins at subzero temperatures is essential to improve storage efficiency, facilitate transportation, and enhance freeze-drying processes. Isochoric cooling, which involves maintaining a fixed volume, is a preferred method for achieving subzero temperatures without freezing aqueous solutions, although it can accelerate the cold-induced cleavage of polypeptides by causing them to unfold. Initially, isochoric chilling was employed to expedite the formulation of proteins, specifically the biological protein disulfide isomerase A1. Various solutions, such as sucrose, glycerol, and L-arginine, were utilized through osmotic methods to significantly enhance the stability of protein isomerase A1 at -20°C, as demonstrated by isochoric cooling over a 700-hour period. For instance, when insulin was subjected to isochoric cooling at 20°C, it experienced a 22% reduction in activity after 15 days. However, a 0.6 M sucrose solution effectively limited insulin degradation (Correia *et al.,* 2020).Isochoric cooling at subzero temperatures has also been observed to induce the aggregation of hemoglobin, which is considered a significant effect in inhibiting freezing stress (Rosa *et al.,* 2013). Moreover, as the temperature decreased, there was an exponential increase in the rate of hemoglobin aggregation, indicating that subzero temperatures can promote cold-mediated aggregation even without freezing stress.

In another study, experimental data on the temperature-pressure relationship during the isochoric freezing of aqueous solutions containing glucose and glycerol, commonly used as cryoprotectants in traditional freezing procedures, were examined. It was found that an increase in pressure during isochoric freezing can be detrimental to biomolecules and restrict the temperature range in which isochoric systems can be employed for preservation, typically up to pressures below 40 MPa. However, the addition of glycerol to saline solutions at various concentrations expanded the range of temperatures at which cryopreservation by isochoric freezing is feasible, such as -11°C with 2M glycerol, -16.5°C with 3M glycerol, and -24.5°C with 4 M glycerol (Beșchea *et al.,* 2021).

1. **Eradication of microorganisms by isochoric freezing**

Generally, in microorganisms, the structure is modified during the process of isohoric freezing. Escherichia coli membranes were found to be disrupted by isochoric cooling at a temperature of –15 °C. This damage resulted in changes in cell size and shape, the production of protrusions, membrane rupture, and the evacuation of intracellular contents (Salinas-Almaguer *et al.,* 2015). Another study found that after 12 hours of freezing, the E. coli population significantly decreased by 2.5 logs at both -15 °C (145 MPa) and -20 °C (186 MPa).After 24 hours at -20 °C, there was a 75% decrease in the number of surviving organisms (Powell-Palm *et al.,* 2018). Additionally, it has been demonstrated that isochoric preservation can kill harmful bacteria like *Listeria Monocytogenes* and *Salmonella Typhimurium*, particularly when performed at -15 °C and 135 MPa for 24 hours. High pressure during isochoric freezing seems to have advantages for the elimination of microbes.

Notably, at -15 °C, isochoric treatment entirely eliminated E. coli due to the bacterial suspension's existence in a metastable and amorphous liquid form, which is obnoxious to bacterial survival. Although some E. coli was observed to be partially killed at -20 °C and -30 °C during the isochoric freezing process due to the formation of ice III, some bacteria tried to conceal themselves inside ice crystals and could potentially replicate after the freezing process (Salinas-Almaguer *et al.,* 2015). Although more research is needed to determine the precise processes underlying the bactericidal effect, the combination of high pressure and low temperature can be attributed to the decreased survival of microbes. The isochoric layout is easy and convenient for use even in home freezers, providing a way to disinfect food products at home.

1. **Consequences of isochoric freezing on enzymes**

It has been discovered that using isochoric freezing dramatically reduces food browning. For instance, potatoes held at -5 °C utilizing isochoric refrigeration showed observable colour changes from those stored at the same temperature using isobaric freezing (Lyu *et al.,* 2017). In most cases, browning in potatoes occurs by the oxidation of phenolic substances by the enzyme polyphenol oxidase (PPO), which produces quinones that eventually polymerize into insoluble melanin, giving food a dark colour. Enzymatic browning results from the release of PPO from the potatoes when the cell membrane is ruptured. Isochoric treatment helps retain cell integrity, which lowers the impact of browning by limiting the release of PPO from potato cell membranes (Lyu *et al.,* 2017). The onset of browning in fresh and thawed potatoes stored under isochoric, isobaric, and individual quick-freezing settings at varied freezing times was examined (Bilbao-Sainz *et al.,* 2020). The presence of ice crystals during food freezing using standard freezing techniques at atmospheric pressure may minimize undesirable enzymatic reactions by removing enzymes from the cell membrane (Năstase et al., 2017). Conventional freezing can harm cell membranes, which can promote enzyme-substrate reactions and result in the degeneration of ascorbic acid. In contrast to isobaric freezing, which results in roughly 63% and 51% losses at -4 °C and -7 °C respectively, isochoric freezing of cherries at the same temperature helps to preserve the ascorbic acid content. Due to the activity of ascorbic acid oxidase, individual quick freezing (IQF) can also reduce the ascorbic acid content of cherries (with a 72% loss) (Bilbao-Sainz *et al.,* 2019).

There was a decrease in ascorbic acid content when potatoes had undergone minimal processing and were kept for four weeks in an isochoric freezing system at a temperature of -3 °C and a pressure of 30 MPa. Freezing the minimally processed potatoes at constant volume possessed around 6.9% of their ascorbic acid content, whereas their counterparts frozen at constant pressure retained about 10.4% of ascorbic acid. Because of the pre-processing steps (peeling, cutting, and freezing), which cause breakage of cells and release of ascorbate oxidase enzymes that increase interactions with ascorbic acid, therefore reducing the amount of ascorbic acid in the isochoric system of potatoes (Bilbao-Sainz *et al.,* 2020).

1. **Application of isochoric freezing on other foods**

The study demonstrates the exploration of isochoric freezing's potential for chicken breast meat. The study involved immersing chicken breast samples in isochoric NaCl solutions with varying concentrations—0 (pure water, PW), 1.5%, and 2.5%—at temperatures of -4°C and -8°C. The investigation aimed to assess how process parameters such as temperature, pressure, and solution concentration affected the quality attributes of the chicken breast samples, including colour, water retention, weight loss, texture, microstructure, and water mobility. The findings revealed that higher NaCl concentrations resulted in a reduction in freezing temperature and pressure. Chicken breast samples treated in PW and 1.5% NaCl solution at -4°C and -8°C exhibited a significant decline in their quality characteristics. Conversely, those treated in the 2.5% solution at -4°C and -8°C did not display any significant differences compared to the control group.These findings suggest the potential to enhance the quality of preserved meats when utilizing isochoric systems (Rinwi *et al.,* 2023). Regarding the impact of different treatment conditions on weight loss, significant reductions in weight loss were observed. Specifically, for freezing at -4°C, the weight losses were 12.18%, 7.2%, and 1.5% with increasing NaCl solution concentrations of 0%, 1.5%, and 2.5%, respectively. Similarly, when considering isochoric freezing at -8°C, the weight losses amounted to 14.17%, 9.62%, and 7.5% for the corresponding NaCl solution concentrations of 0%, 1.5%, and 2.5%. The notable weight loss observed in samples treated with pure water (PW) may be attributed to the formation of large ice crystals, leading to structural damage within the muscle bundle. This, in turn, resulted in drip loss and muscle fiber dehydration. In terms of color parameters, samples treated with a 0% NaCl solution at both -4°C and -8°C showed significant increases in lightness (L\*) and yellowness (b\*) values, along with significant decreases in redness (a\*) values. Conversely, L\*, b\*, and a\* values from samples treated with 1.5% NaCl solution at -4°C and those treated with 2.5% NaCl solution at both -4°C and -8°C exhibited no significant differences when compared to the control. Regarding texture characteristics, significant increases in hardness were observed in samples treated with increasing NaCl solution concentrations of 0%, 1.5%, and 2.5%. These values were 42.91, 50.32, and 55.32 N for freezing at -4°C and 25.58, 39.82, and 52.86 N for freezing at -8°C, respectively, compared to 55.52 N for the fresh sample (control). Conversely, slight differences were noted in springiness, cohesiveness, and chewiness between the samples treated with 1.5% and 2.5% NaCl solutions at both -4°C and -8°C. In contrast, samples treated with PW exhibited significant increase in springiness, cohesiveness, and chewiness, with values varying across different freezing conditions. These observed changes in texture characteristics may be attributed to underlying molecular mechanisms involving protein denaturation, particularly myosin and actin. These proteins play pivotal roles in determining meat texture.

Top of Form

One of the studies delved into the conformational structures of myofibrillar proteins during the isochoric freezing process applied to chicken breasts at three distinct temperatures: -4°C, -8°C, and -12°C. Experiments utilizing a 2.5% NaCl solution yielded noteworthy findings. Samples subjected to -4°C and 25 MPa showed no substantial impact on myofibrillar protein structure. In contrast, those treated at -8°C and 60 MPa exhibited significant alterations in protein properties, encompassing dityrosine, solubility, and sulfhydryl, suggesting a partial recovery of the samples. However, samples treated at -12°C and 85 MPa revealed complete disruption of the myofibrillar protein structure. Additionally, the evaluation of myofibrillar sarcomeres corroborated these findings, confirming that freezing at -4°C effectively preserved the protein structure in chicken breast meat. These insights offer potential avenues for preserving meat using isochoric freezing within the food industry (Rinwi *et al.,* 2023).

Top of Form

Fish and fish-based products play a pivotal role in global nutrition and food security, offering a rich source of essential nutrients and micronutrients like high-quality protein, omega-3 polyunsaturated fatty acids (PUFA), and vitamins (A, B, and D), as well as essential minerals like calcium, phosphorus, zinc, and iron (Bene *et al.,* 2016). The aim of this study was to investigate how isochoric freezing affects the freshness of tilapia fish in terms of attributes such as color, texture, thiobarbituric acid reactive substances (TBARS), and total volatile basic nitrogen (TVB-N) content. Additionally, it sought to compare the outcomes of isochoric freezing (-3°C/37 MPa) with other preservation methods, including chilling (5°C), super-chilling (-3°C), and freezing (-20°C). Under isochoric freezing conditions, the tilapia muscle exhibited a slight increase in mass (less than 4%). Isochoric freezing also led to a minor yet significant rise in water content, while salt content remained unchanged. In contrast, super-chilling and freezing resulted in mass loss due to drip loss. Fresh tilapia fillets displayed a slightly translucent appearance with a hint of reddish color. Isochoric freezing caused a noteworthy increase in the L\* value and a modest but significant decrease in the a\* value. The yellowness parameter b\* remained unaltered. Chilling did not affect the L\* value within the first 7 days but significantly increased it after 14 days. Additionally, a\* slightly decreased with chilling, while b\* remained constant. Super-chilling led to increases in both L\* and b\* values while reducing a\* values. Freezing produced color changes similar to those observed in isochoric freezing. The rise in L\* value in isochoric samples was attributed to brine absorption in muscle tissue, a phenomenon also observed in fish fillets immersed in 0.2% NaCl brine at 5°C for one day. Fresh tilapia muscle exhibited a tightly arranged structure with minimal spacing between muscle fiber bundles. Muscle fibers were evenly distributed, featuring regular polygonal shapes and surrounded by thin connective tissue layers. Isochoric samples displayed homogeneous muscle fiber bundles with polygonal shapes, enveloped by collagenous fibrils. However, noticeable gaps were visible between the cells. The isochoric freezing process retained the structural integrity of muscle fibers by maintaining comparable osmolality between extracellular and intracellular environments. This helped prevent cell dehydration and solute damage, a common occurrence in freezing at atmospheric pressure (Nӑstase *et al.,* 2017). Regarding textural properties, chilled, super-chilled, and frozen samples all exhibited decreases across all five measured properties. In contrast, isochoric-frozen fish displayed increased cohesiveness, signifying improved resistance to repeated deformations. While all samples exhibited notable reductions in hardness, chewiness, and gumminess compared to the fresh fillet, the isochoric sample had the highest values for both springiness and texture properties. These results indicate that isochoric frozen fish closely approximated the texture properties of fresh fillets, corroborating the findings from micrographs of the muscle tissues (Bilbao-Sainz *et al.,* 2020).

The research aimed to explore the fundamental processes driving moisture movement in chicken breast meat under three distinct isochoric treatment conditions: direct immersion freezing (DIF), vacuum pack freezing (VPF), and vacuum immersion pack freezing (VIPF). The study investigated how these treatments, conducted at -4°C using a 2.5 g/dL sodium chloride solution, and influenced the overall quality of the chicken breast meat. The study revealed that various moisture transfer mechanisms, including diffusion and infusion, played a pivotal role in altering the distribution and mobility of water within the meat. These changes had significant and measurable impacts on several attributes, including color, water holding capacity (WHC), pH, cooking loss, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), solubility, and the structural integrity of the DIF-treated samples. Conversely, VIPF-treated samples exhibited only slight effects, with notable differences observed primarily in pH and WHC. VPF-treated samples, on the other hand, did not show any significant deviations from the characteristics of fresh samples. In conclusion, this study suggests that the isochoric freezing protocol can be tailored to suit specific sample types and desired outcomes. It also offers valuable theoretical and empirical insights for the development of customized isochoric freezing protocols (Rinwi *et al.,* 2023).

1. **Energy consumption during the process**

To assess the potential for energy conservation, researchers calculated the ratios of energy required to freeze identical masses in both isochoric and isobaric systems. It has been found that an isochoric system requires much less energy than an isobaric system with an identical mass. Energy consumption has been reduced due to two physical phenomena: a decrease in the total frozen mass and the temperature-dependency of water on the latent heat of fusion. Only a part of the mass in an isochoric system will actually freeze at any subfreezing temperature higher than the triple point, thus reducing the overall energy needed for ice fusion. Nonetheless, the energy needed to freeze this restricted portion is also lower compared to what would be necessary in an isobaric system. This difference arises from the fact that the latent heat of fusion diminishes as the temperature drops. This particular temperature-dependent characteristic does not offer advantages to isobaric systems because, as previously emphasized, they undergo the entire phase transition at the atmospheric freezing point. In contrast, in an isochoric system, the freezing point diminishes as the phase transition advances, leading to decreased energy requirements for freezing. When it comes to latent heat, conventional isobaric systems tend to maximize the energy required for freezing, while isochoric systems inherently minimize this energy demand. As previously mentioned, the primary objective of isochoric cold storage at subfreezing temperatures is to safeguard preserved food from ice formation. In practical food storage scenarios, the isochoric system aims to protect only a portion of the preserved food from ice-related damage. Although this situation is relevant in industrial contexts, a further comparison is needed to assess the energy required to protect the entire mass of food matter. To address this, a novel isochoric system is proposed, which introduces two mass-related concepts: the "food mass," representing the portion of food matter to be shielded from ice formation, and the "design mass," signifying the total system mass needed to ensure that a portion equal to the food mass remains unfrozen (Zhao *et al.,* 2021).In order to reduce the size of the ice crystals, industrial food products are first frozen at extremely low temperatures, and then they are stored at freezing temperatures. This technique requires a large amount of energy. According to thermodynamic analyses, the preservation of fish or meat in an isochoric system at -5 °C uses 70% less energy than conventional freezing. Even more energy can be saved by storing foods such as fruits and berries with high sugar content. Isochoric storage has the potential to lower energy usage on an industrial scale as the ice does not form inside the food. Without requiring significant infrastructural upgrades or the loss of equipment, isochoric systems can be installed by altering existing large- scale freezers to enhance efficiency. Additionally, the simple construction of isochoric systems makes their use a practical and affordable alternative.

**2. CHALLENGES**

Preservation through isochoric methods presents several benefits in contrast to traditional freezing techniques. Nevertheless, certain aspects related to research and commercialization demand more concentrated effort and consideration. There have been cases in which the application of elevated pressures in isochoric systems has resulted in adverse outcomes, such as compromising the viability of organs during the preservation of a rat's heart (Wan *et al.,* 2018).This emphasizes the need for methods that are optimized and consider both pressure and temperature for a variety of food freezing purposes. The specific mechanisms causing the late-onset of enzymatic browning and a drop in ascorbic acid content in vegetables with minimum processing require additional investigation (Bilbao-Sainz *et al.,* 2020). Similarly, there is an inadequate explanation of the basic theories behind the bactericidal effects of isochoric freezing. Theoretical and experimental investigations are necessary to clarify the super-cooling stability of isochoric systems with regard to equipment design and process control. These aspects should be explored further before deeming the technology suitable for widespread industrial applications.

1. **CONCLUSION**

The food industry employs various preservation methods to maintain food quality and reduce storage losses. There is a growing need for innovative and cost-effective approaches due to the impact of food preservation on nutritional aspects. Isochoric freezing has emerged as a promising method, demonstrating benefits such as delayed ripening without compromising nutritional parameters, maintaining optimal storage conditions without altering water activity, minimizing the use of cryoprotectants, and inhibiting the growth of food spoilage microorganisms under controlled temperature and fixed volume conditions. Moreover, the preservation of food macromolecules during isochoric treatments signifies the potential to provide healthy and nutritious foods.

**REFERENCES**

Alonso, J., Rodriguez, T., & Canet, W. (1995). Effect of calcium pretreatments on the texture of frozen cherries. Role of pectinesterase in the changes in the pectic materials. *Journal of Agricultural and Food Chemistry*, *43*(4), 1011-1016.

Alonso, J., Tortosa, M. E., Canet, W., & Rodríguez, M. T. (2005). Ultrastructural and changes in pectin composition of sweet cherry from the application of prefreezing treatments. *Journal of food science*, *70*(9), E526-E530..

Basak, S., & Ramaswamy, H. S. (1998). Effect of high pressure processing on the texture of selected fruits and vegetables. *Journal of Texture Studies*, *29*(5), 587-601.

Béné, C., Arthur, R., Norbury, H., Allison, E. H., Beveridge, M., Bush, S., ... & Williams, M. (2016). Contribution of fisheries and aquaculture to food security and poverty reduction: assessing the current evidence. *World development*, *79*, 177-196.

Beșchea, G. A., Câmpean, Ş. I., Tăbăcaru, M. B., Şerban, A., Rubinsky, B., & Năstase, G. (2021). Glucose and glycerol temperature-pressure correlations for the design of cryopreservation protocols in an isochoric system at subfreezing temperature. *Biochemical and Biophysical Research Communications*, *559*, 42-47.

Bilbao-Sainz, C., Sinrod, A. J., Williams, T., Wood, D., Chiou, B. S., Bridges, D. F., ... & McHugh, T. (2020). Preservation of tilapia (Oreochromis aureus) fillet by isochoric (constant volume) freezing. *Journal of Aquatic Food Product Technology*, *29*(7), 629-640.

Bilbao-Sainz, C., Chiou, B. S., Takeoka, G., Williams, T., Wood, D., Powell-Palm, M. J., ... & McHugh, T. (2022). Isochoric freezing and isochoric supercooling as innovative postharvest technologies for pomegranate preservation. *Postharvest Biology and Technology*, *194*, 112072..

Bilbao‐Sainz, C., Sinrod, A. G., Dao, L., Takeoka, G., Williams, T., Wood, D., & McHugh, T. (2020). Preservation of spinach by isochoric (constant volume) freezing. *International Journal of Food Science & Technology*, *55*(5), 2141-2151.

Bilbao-Sainz, C., Sinrod, A. J., Dao, L., Takeoka, G., Williams, T., Wood, D., ... & McHugh, T. (2021). Preservation of grape tomato by isochoric freezing. *Food Research International*, *143*, 110228.

Bilbao-Sainz, C., Sinrod, A. J., Williams, T., Wood, D., Chiou, B. S., Bridges, D. F., ... & McHugh, T. (2020). Preservation of tilapia (Oreochromis aureus) fillet by isochoric (constant volume) freezing. *Journal of Aquatic Food Product Technology*, *29*(7), 629-640.

Bilbao-Sainz, C., Sinrod, A., Powell-Palm, M. J., Dao, L., Takeoka, G., Williams, T., ... & McHugh, T. (2019). Preservation of sweet cherry by isochoric (constant volume) freezing. *Innovative food science & emerging technologies*, *52*, 108-115.

Câmpean, Ș. I., Beșchea, G. A., Tăbăcaru, M. B., Scutaru, L. M., Dragomir, G., Brezeanu, A. I., ... & Năstase, G. (2023). Preservation of black grapes by isochoric freezing. *Heliyon*, *9*(7).

Cantwell, M., Nie, X., & Hong, G. (2009, April). Impact of storage conditions on grape tomato quality. In *6th ISHS postharvest symposium, Antalya, Turkey* (Vol. 8)..

Correia, C., Tavares, E., Lopes, C., Silva, J. G., Duarte, A., Geraldes, V., ... & Melo, E. P. (2020). Stability of protein formulations at subzero temperatures by isochoric cooling. *Journal of pharmaceutical sciences*, *109*(1), 316-322.

Gould, G. W. (1996). Methods for preservation and extension of shelf life. *International journal of food microbiology*, *33*(1), 51-64.

Kong, C. H., Hamid, N., Ma, Q., Lu, J., Wang, B. G., & Sarojini, V. (2017). Antifreeze peptide pretreatment minimizes freeze-thaw damage to cherries: An in-depth investigation. *Lwt*, *84*, 441-448..

Lisiewska, Z., & Kmiecik, W. (2000). Effect of storage period and temperature on the chemical composition and organoleptic quality of frozen tomato cubes. *Food Chemistry*, *70*(2), 167-173.

Lyu, C., Nastase, G., Ukpai, G., Serban, A., & Rubinsky, B. (2017). *Isochoric refrigeration of food products* (No. e2740v1). PeerJ Preprints..

Năstase, G., Lyu, C., Ukpai, G., Şerban, A., & Rubinsky, B. (2017). Isochoric and isobaric freezing of fish muscle. *Biochemical and Biophysical Research Communications*, *485*(2), 279-283.

Patras, A., Brunton, N. P., Da Pieve, S., & Butler, F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science & Emerging Technologies*, *10*(3), 308-313.

Powell-Palm, M. J., Preciado, J., Lyu, C., & Rubinsky, B. (2018). Escherichia coli viability in an isochoric system at subfreezing temperatures. *Cryobiology*, *85*, 17-24..

Rinwi, T. G., Ma, J., & Sun, D. W. (2023). Effects of isochoric freezing on myofibrillar protein conformational structures of chicken breasts. *LWT*, *181*, 114768.

Rinwi, T. G., Sun, D. W., Ma, J., & Wang, Q. J. (2023). Effects of isochoric freezing on freezing process and quality attributes of chicken breast meat. *Food Chemistry*, *405*, 134732.

Rinwi, T. G., Sun, D. W., Ma, J., & Wang, Q. J. (2023). Underlying mechanisms and molecular interactions of isochoric freezing on enhancing the freshness of chicken breast meat. *LWT*, 115305.

Rosa, M., Lopes, C., Melo, E. P., Singh, S. K., Geraldes, V., & Rodrigues, M. A. (2013). Measuring and modeling hemoglobin aggregation below the freezing temperature. *The Journal of Physical Chemistry B*, *117*(30), 8939-8946..

Salinas-Almaguer, S., Angulo-Sherman, A., Sierra-Valdez, F. J., & Mercado-Uribe, H. (2015). Sterilization by cooling in isochoric conditions: the case of Escherichia coli. *Plos one*, *10*(10), e0140882.

Wan, L., Powell-Palm, M. J., Lee, C., Gupta, A., Weegman, B. P., Clemens, M. G., & Rubinsky, B. (2018). Preservation of rat hearts in subfreezing temperature isochoric conditions to–8 C and 78 MPa. *Biochemical and biophysical research communications*, *496*(3), 852-857.

Zdunek, A., Gancarz, M., Cybulska, J., Ranachowski, Z., & Zgorska, K. J. I. A. (2008). Turgor and temperature effect on fracture properties of potato tuber [Solanum tuberosum cv. Irga]. *International agrophysics*, *22*(1), 89-97.

Zhao, Y., Bilbao-Sainz, C., Wood, D., Chiou, B. S., Powell-Palm, M. J., Chen, L., ... & Rubinsky, B. (2021). Effects of isochoric freezing conditions on cut potato quality. *Foods*, *10*(5), 974.

Zhao, Y., Powell-Palm, M. J., Wang, J., Bilbao-Sainz, C., McHugh, T., & Rubinsky, B. (2021). Analysis of global energy savings in the frozen food industry made possible by transitioning from conventional isobaric freezing to isochoric freezing. *Renewable and Sustainable Energy Reviews*, *151*, 111621.