

ISOCHORIC FREEZING: AN INNOVATIVE TECHNOLOGY FOR FRUITS AND VEGETABLES PRESERVATION

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

Authors

Charu Bisht

Research Scholar,
Department of Food Science & Technology,
Govind Ballabh Pant University of
Agriculture & Technology,
Pantnagar, U. S. Nagar, Uttarakhand, India.
charubisht3096@gmail.com

Deeksha Semwal

Research Scholar,
Department of Food Science & Technology,
Govind Ballabh Pant University of
Agriculture & Technology,
Pantnagar, U. S. Nagar, Uttarakhand, India.

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 $-18\text{ }^{\circ}\text{C}$. 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 ($0\text{ }^{\circ}\text{C}$) during the pre-cooling phase. The super-cooling phase is brought on by further heat removal to temperatures below $10\text{ }^{\circ}\text{C}$. 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.

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

- 2. 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.
- 3. 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 °C)	Varies (-18 °C 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

- 4. 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.

II. EFFECT ON NUTRITIONAL CHARACTERISTICS OF FRUITS

- 1. Ascorbic Acid Content:** Ascorbic acid, commonly known as vitamin C, is primarily derived from fruit juices and is a crucial antioxidant found in various plant sources, particularly fruits. Fruits serve as a major dietary source of ascorbic acid for humans and play a significant role in fruit ripening, stress resistance, growth regulation, and postharvest storage. However, ascorbic acid is sensitive to factors like oxygen exposure, pH changes, temperature fluctuations, and pressure, making its preservation a top priority for food manufacturers when processing fruit and vegetable products. In a specific study, the research focused on the potential of isochoric freezing as a method to preserve the ascorbic acid content in tomatoes. Traditional freezing processes often result in texture degradation, color alterations, and nutrient loss in tomatoes. Fresh grape tomatoes were initially found to contain 196 ± 21 mg/100 g (dry basis) of ascorbic acid. Under isochoric freezing conditions, tomatoes managed to maintain their ascorbic acid levels for a period of four weeks. During the first two weeks, there was a 17% reduction in ascorbic acid content, but no further losses occurred after this point. In contrast, isobaric freezing led to

a substantial reduction in ascorbic acid, with only 10% of the initial content remaining at the end of storage (Bilbao-Sainz *et al.*, 2021).

Fresh Rainier cherries had an original total ascorbic acid content of 5.3 ± 0.8 mg/100 g. Isochoric conditions successfully preserved the ascorbic acid content in cherries, even when subjected to a pressure increase from 30 MPa to 62 MPa. It was observed that hydrostatic pressure did not impact covalent bonds or alter low molecular weight compounds like vitamins, as supported by previous research. In contrast, other freezing methods, such as IQF and isobaric freezing, caused significant losses in ascorbic acid content. This included a 72% loss in the case of IQF, a 63% loss at 4 °C under isobaric freezing, and a 51% loss at 7 °C under isobaric freezing. Additionally, there was a 12% drop in ascorbic acid content as soon as cherries were frozen at -18 °C. The likely cause of this degradation was enzymatic oxidation by ascorbic acid oxidase, which occurs in the presence of oxygen. Enzymatic reactions, though slowed down in frozen products, still take place when non-frozen water is present. Notably, isobaric and IQ freezing increased these enzymatic activities as the freezing process damaged cell membranes, promoting enzyme-substrate interactions during thawing, unlike isochoric freezing (Bilbao-Sainz *et al.*, 2019).

Another study examined the effects of isochoric supercooling at -2.5 °C and isochoric freezing at -2.5 °C/12 MPa on the quality of whole pomegranate arils (cv. "Wonderful") and fresh-cut arils stored for 30 days. These were compared with cold storage at 5 °C/95% relative humidity and isobaric freezing at 2.5 °C/0.1 MPa. Initially, fresh pomegranate arils contained 12.68 mg of ascorbic acid per 100g of juice. The results showed that isochoric frozen samples exhibited the highest increase in ascorbic acid concentration, primarily due to pressure-induced impregnation. The increase in ascorbic acid concentration in isochoric supercooled samples might be attributed to the movement of ascorbic acid into the arils as cell membranes broke down during storage. Conversely, ice crystals likely damaged the structural integrity of cellular compartments in isobaric frozen arils. The loss of semi-permeability in cellular membranes resulted in mass transfer between the arils and the surrounding 16% sucrose/0.5% ascorbic acid solution (Bilbao-Sainz *et al.*, 2022).

- 2. Antioxidant Activity:** Antioxidant activity plays a crucial role in preserving the integrity and functionality of cells by effectively eliminating free radicals, inhibiting lipid peroxidation, and preventing oxidative damage. It underpins numerous biological processes related to combatting conditions like cancer, inflammation, and the aging process. Furthermore, antioxidant activity is associated with the prevention of chronic diseases such as cancer, diabetes, and cardiovascular issues. Hence, it is imperative to conduct comprehensive research on natural antioxidants found in fruits and vegetables.

Research has shown that antioxidant compounds have the potential to enhance human health by neutralizing free radicals and thwarting the oxidative processes that contribute to degenerative diseases. In cherries, the high antioxidant activity is primarily attributed to anthocyanins, flavonoids, and total phenolic content. Although slightly lower than previously reported values of 3.3 ± 0.1 mg TE.g-1 in cherry flesh and 6.8 ± 0.4 mg TE.g-1 in cherry peel, fresh cherries exhibit an antioxidant activity of 1.9 ± 0.1 mg TE.g-1. The freezing process at -4°C in isochoric conditions had a minimal impact on

antioxidant activity. However, cherries stored under isochoric conditions at -7°C showed a seemingly higher antioxidant activity, whereas those frozen under isobaric conditions experienced a slight decrease in antioxidant activity (Bilbao-Sainz *et al.*, 2019).

Fresh tomatoes were found to possess an antioxidant activity of 16.6 ± 2.3 mg TE/g (dry basis). The majority of this antioxidant activity is attributed to soluble antioxidants, primarily ascorbic acid and soluble phenolics, which contribute 92% of the total antioxidant activity. In contrast, lipophilic antioxidants, mainly lycopene and lipophilic phenolics, constitute only 8% of the total. Freezing tomatoes at -2.5°C in isochoric conditions did not significantly alter their antioxidant activity. However, in samples preserved by other methods, a progressive decline in antioxidant activity occurred over time due to the degradation of bioactive compounds (Bilbao-Sainz *et al.*, 2021).

The antioxidant activity in pomegranate fruits primarily stems from hydrolysable tannins (Punicalagins and Punicalins) and phenolic acids, such as ellagic acid. Fresh pomegranate arils exhibited an antioxidant activity of 3.96 ± 0.37 mg TEg⁻¹ (DPPH assay) and 3.94 ± 0.34 mg TEg⁻¹ (ABTS^{•+} assay). During cold storage, the antioxidant activity in arils from whole pomegranates declined. Notably, regardless of the postharvest preservation method, the antioxidant activity in fresh-cut arils was lower than that in whole pomegranate arils. The highest antioxidant activity was observed in isochoric supercooled and cold-stored arils, while the lowest antioxidant activity was found in isochoric frozen and isobaric frozen arils (Bilbao-Sainz *et al.*, 2022).

III. EFFECT ON MECHANICAL PROPERTIES OF FRUITS

The mechanical properties of fruits and vegetables play a crucial role in determining the texture of food products. Understanding the mechanical properties can aid in selecting appropriate processing methods, determining the optimal harvest time, and choosing detachment techniques. It also helps in anticipating potential damage during the collection, processing, and storage of these foods. Cold storage, isochoric freezing, and isobaric freezing have all affected the textural qualities of fruits, particularly pomegranate arils. Isochoric frozen arils experienced the most significant texture degradation, with a 22% loss in hardness, 41% in crispiness, and 37% in crunchiness, indicating pressure-related physiological issues. Similarly, cold-stored arils lost 22% of their hardness, 29% of their crispiness, and 30% of their crunchiness due to ongoing metabolic processes. Notably, isobaric frozen arils underwent substantial textural changes, losing 15% in hardness, 7% in crispiness, and 17% in crunchiness on average. The softening and reduced crunchiness could be attributed to the immersion of arils in the aqueous solution. The most considerable texture loss resulted from the development of ice during isobaric freezing, which weakened cell membranes and walls, leading to cell lysis and subsequent loss of water and cellular components, thereby softening the arils (Bilbao-Sainz *et al.*, 2022).

Consumers often make their initial purchasing decisions based on a product's appearance, but repetitive consumption are influenced by factors like texture. To enhance consumer appeal, frozen cherries should closely resemble the texture of fresh cherries. The best mechanical qualities were found in cherries preserved under isochoric conditions. When compared to fresh cherries, cherries stored at -4°C under isochoric conditions showed no

significant variations in maximum force, elasticity modulus, or strain fracture values. High hydrostatic pressures can damage cell structures and reduce cell membrane permeability, leading to a slight decrease in sample firmness and rigidity when lowering the temperature from -4°C to -7°C . In contrast, IQ freezing and isobaric freezing resulted in the most substantial changes in textural characteristics. The firmness of cherries subjected to these freezing methods was significantly lower than that of fresh cherries, indicating an impact on the strength of cell walls and membranes. Both freezing methods caused a significant sample softening due to cell lysis and subsequent loss of water and cellular components. Previous research has also indicated that thawed cherries become mushy after prolonged storage at -20°C , and the hardness and elasticity modulus values of IQF cherries decrease. Intracellular pectin leakage, which disrupts calcium bridges and loosens cell tissue, has been identified as a key factor in the loss of firmness. The results suggested that preserving cherries under isochoric conditions results in a texture similar to fresh Rainier cherries, making it a superior option compared to IQ freezing and preservation under isobaric conditions (Bilbao-Sainz *et al.*, 2019).

IV. EFFECT ON VISUAL AND COLOUR CHARACTERISTICS OF FRUITS

The color of fruit, in addition to its flavor and taste, is a significant biochemical characteristic that greatly influences consumers' preferences, particularly in the case of grapes. When pomegranate arils were stored as whole fruits using isochoric supercooling or isochoric freezing, minor differences in color values (E^*) were observed, as all color parameters remained relatively stable. However, during cold storage, a noticeable change occurred as it significantly reduced the color tone (hue angle, h°), and isobaric freezing led to a substantial decrease in h° and yellowness (b^*) attributes. The redness (a^*) values of freshly cut arils remained largely unaffected by various postharvest treatments, but b^* and h° levels experienced significant reductions. The coloration of pomegranate arils is primarily attributed to anthocyanin pigments. Refrigerated samples displayed diminished visual appeal due to microbial degradation, surface mycelia growth, and mild browning, a result of the oxidation of phenolic compounds. Isochoric supercooling preserved the vibrant color of fresh arils, although the color tone appeared slightly faded. In contrast, isochoric or isobaric freezing caused the distinctive vivid color and color tone of pomegranate arils to disappear, possibly due to pigment movement, enzymatic browning, and anthocyanin degradation resulting from damaged cells (Bilbao-Sainz *et al.*, 2022).

Grape tomatoes have a tendency to lose weight during storage, leading to shriveling and dehydration after three weeks. Nearly all tomatoes stored at 10°C were of lower quality, which aligns with the findings of Cantwell, Nie, and Hong (2009), who noted that grape tomatoes maintained their visual appeal for 12 days at 10°C . In terms of appearance, IQF and isobaric frozen tomatoes had the least appeal, with the peel separating from the tomato pulp and appearing mushy. Compared to IQF, isobaric freezing had a more adverse impact on tomato appearance. Isochoric freezing and cold storage had minimal effects on the $L^* a^* b^*$ color values, with low ΔE^* values indicating that the color of these tomatoes remained consistent with the fresh sample throughout the preservation period. In contrast, IQF and isobaric samples exhibited a more yellowish hue (higher b^* values) compared to fresh samples, with this effect being more pronounced for IQF samples. Furthermore, the isobaric and IQF samples displayed higher ΔE^* values compared to the cold-stored and isochoric frozen tomato samples. The freezing of tomatoes and tomato products often results in color

changes, characterized by increased yellowness and lightening of color. This is attributed to a reduction in carotenoid concentration due to enzymatic oxidation. IQF samples had a higher b^* value compared to isobaric samples, likely due to greater carotenoid oxidation caused by air circulation during frozen storage at -20°C in IQF samples (Bilbao-Sainz *et al.*, 2021). When cherries were isochorically frozen, their color appeared darker, and there was a slight increase in a^* (redness) and b^* (yellowness) values. This may have occurred due to the sucrose solution filling the tissue pores, giving the fruit a translucent appearance. Notably, hydrostatic pressure had no apparent effect on the degradation of β -carotene, the primary compound responsible for the yellow color of Rainier cherries, and phenolic compounds at -4°C and -7°C , as there were no significant differences in color. In contrast, isobaric and IQ frozen samples displayed a considerable reduction in L^* and b^* values, with increased a^* values. This reduction in color attributes was more pronounced as temperatures dropped below freezing, likely due to enzymatic oxidation of β -carotene. Additionally, enzymatic browning likely occurred through the action of polyphenol oxidases and peroxidases, facilitated by cell membrane damage during freezing. The color variations observed in cherries were likely the result of interactions between enzymes and substrates accelerated by membrane degradation during freezing. The lowest chromatic difference in cherries was observed in isochoric preservation at -4°C and -7°C , while the highest chromatic difference was observed in IQ freezing (32%). Chromatic differences of 17% and 30% were found in samples frozen at -4°C and -7°C in an isobaric system, respectively.

The color of black grapes is primarily influenced by anthocyanins and flavonoids, which are sensitive to temperature and environmental conditions. The formation of ice crystals during conventional freezing can harm grape cells and cell walls, leading to color release and changes. In the case of black grapes, isochoric preservation without maintaining constant pressure (constant volume) is essential for preserving their quality. Isochoric preservation effectively reduces ice crystal formation and cellular damage, thereby helping maintain the Brix level and color of black grapes. (Campean *et al.*, 2023).

V. EFFECT ON STRUCTURAL PROPERTIES OF FRUITS

The application of cryo-SEM images at the cellular level is valuable for comprehending how freezing impacts the structure of cherries. Fresh cherry cells appear firm and densely packed, with some intercellular spaces containing air. In cherries that underwent isochoric freezing at -4°C , the cell walls and membranes remained intact, and the spaces between cells appeared filled with an external sugar solution, but the overall structural integrity of the cherry was unaffected. However, when cherries were subjected to isobaric freezing at -4°C or -7°C , significant structural alterations occurred. Fluid filled the intercellular gaps due to the mobility of cellular components, and some cells appeared loose with wide intercellular spaces, likely due to the absence of intracellular pectin. Dehydration caused cell walls to fold and buckle, leading to severe tissue damage. In the case of IQ freezing, some cell walls exhibited irregular forms and appeared lighter, indicating damage and the release of intracellular content, making it difficult to distinguish between intracellular and extracellular spaces as both were filled with water and particulates (Bilbao-Sainz *et al.*, 2019).

After a 4-week preservation period, cryo-SEM images were used to illustrate the impact of different preservation methods on tomato cells. The tomato peel consists of a thin

layer of epidermal cells and several layers of relatively small, flattened cells. Parenchyma cells, which are polyhedral and come in various sizes and shapes, are separated by numerous intercellular gaps. In tomatoes preserved isochorically, the tissue closely resembled the fresh sample, with no ice crystal formation and low processing pressures preserving cell integrity. While intercellular gaps seemed fluid-filled, the cells themselves remained unharmed. Micrographs revealed corrugation, folding of cells, and a reduction in intercellular gaps, likely due to water loss from senescence and storage processes. In parenchyma tissue from IQF samples, some cells appeared to have lost their cellular structure entirely, while others retained it, possibly aided by the rapid freezing process generating numerous small ice crystals to preserve some cell compartments. In contrast, tomatoes preserved under isobaric conditions suffered extensive histological damage, primarily due to the formation of large ice crystals resulting from slow freezing, causing osmotic imbalances and mechanical damage (Bilbao-Sainz *et al.*, 2021).

VI. ISOCHORIC FREEZING OF VEGETABLES

Finding effective preservation techniques, that extend vegetables shelf life whilst retaining 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. Frozen storage causes a slow, cumulative, and irreversible loss of fresh-like characteristics and overall quality, therefore satisfy the industrial needs in an economical way. Because temperature fluctuations in isochoric systems lead to phase changes rather than sensible temperature changes, food items stored in these systems will experience fewer temperature variations during transport and storage. This suggests that the food enterprises may take advantage of isochoric freezing capabilities without significantly altering the existing refrigeration infrastructure.

1. Effect on Nutritional Composition of Vegetables: Spinach has high antioxidant activity because of the chlorophyll, total soluble phenols and ascorbic acid present in comparatively higher concentration. All samples revealed a decrease in ascorbic acid concentration, chlorophyll content, total soluble phenolic content, and antioxidant activity, albeit the amount of loss varied depending on the freezing process. The nutritional content of the isochoric samples was usually higher than that of the isobaric samples. Additionally, the isobaric sample that was immersed in a solution had more nutrients than the isobaric vacuum- packed sample. Frozen spinach had the least amount of nutrients in comparison to fresh spinach.

Ascorbic acid levels in fresh spinach have been estimated to be 31.1 - 2.9 mg/100 g (w.b.). The isochoric sample had 32% ascorbic acid after the first day, but just 10% after a week. On the other hand, the isobaric sample immersed in a solution managed to retain 15% of its ascorbic acid content after 1 day, with no additional losses observed after 7 days. However, the isobaric vacuum-packed sample experienced a further 10% reduction in ascorbic acid compared to the isobaric sample immersed in the solution. Notably, the commercially frozen sample had the lowest level of ascorbic acid content, measuring at 1.43 ± 0.10 mg g⁻¹. The antioxidant activity in fresh spinach leaves was

found to be 2.02 mg TE/100g (w.b.), equivalent to 337.0 ± 7.3 mg TE/g. All frozen samples demonstrated a decline in antioxidant activity over time. In particular, the isochoric sample displayed the smallest decrease in antioxidant activity, with a reduction of 54% after 1 day and 75% after 7 days. Conversely, 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. Remarkably, the isobaric vacuum-packed sample showed reductions of 82% and 88% after one and 7 days, respectively. The commercially frozen spinach exhibited the lowest antioxidant activity, measuring at 23.1 ± 5.5 mg TE/g (Bilbao-Sainz *et al.*, 2020).

In a study, potato cylinders underwent isochoric freezing, and researchers used a full factorial approach to explore the effects of various processing methods (immersion in water, vacuum packaging, or immersion in an ascorbic acid solution), different freezing temperatures and pressures (-3 °C/37 MPa, -6 °C/71 MPa, -9 °C/101 MPa, and -15 °C/156 MPa), and two different compression rates (below 0.02 and above 0.16 MPa/s) on the quality of frozen potatoes when they were thawed. The observed changes in mass ranged from a 14% increase to an 11% decrease, depending on the specific isochoric freezing conditions used. Notably, when potatoes were immersed in water and frozen at -3 °C, -6 °C, and -9 °C, they gained mass, with the mass gain decreasing from 13.6% at -3 °C to 9.2% at -9 °C. However, at the lowest freezing temperature of -15 °C, the potato samples lost 10.9% of their mass due to various factors. The mass increase was mainly due to water absorption, driven by differences in osmotic potential between the potato cells and their surroundings. 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 increased cell damage at higher pressures. Additionally, potato samples immersed in an 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. 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 highlighted that lower compression rates resulted in less cellular damage for samples processed in an isotonic solution (Zhao *et al.*, 2021).

The increased demand for more convenient food options has led to the expansion of minimally processed potato products. This study investigated 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 revealed that isochoric freezing (at -3 °C/30 MPa) produced better outcomes with lower drip loss, reduced volume shrinkage, and 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, TSP values increased by 81% after 7 days, with no significant changes observed at longer freezing times. This increase in phenolic content during isochoric freezing might be due to heightened phenylalanine ammonia-lyase activity resulting from cell injury, leading 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 appeared 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.

- 2. 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 considering isochoric frozen potato cubes, there was a noticeable increase in maximum stress after 2 weeks. This increase in texture, often observed in response to high-pressure processing, is believed to be due to the activity of pectinmethylesterase and the densification of cellular structure, resulting from the removal of air from the tissue, as suggested by Basak and Ramaswamy (1998). However, the elastic modulus of isochoric frozen potatoes declined by 39% after 3 weeks. In contrast, isobaric and individually quick-frozen (IQF) treated potatoes did not fracture during compression tests due to their loss of rigidity. These samples displayed a significant reduction in both maximum stress and elastic modulus after just 1 week. Specifically, the maximum stress of thawed potatoes decreased by approximately 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 changes 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 softening (Zdunek, Gancarz, Cybulska, Ranachowski, and Zgórska, 2008).

The objective of the study was to evaluate the preservation of baby-leaf spinach through isochoric freezing. Among the various freezing methods tested, the isochoric sample demonstrated the most favorable mechanical properties. These samples experienced a slight reduction in rigidity over time but maintained their crispiness, as indicated by their short distance at break and peak force values, which closely resembled

those of fresh spinach. Conversely, the isobaric sample immersed in a solution displayed a significant decrease in elastic modulus, signalling a more elastic texture and a loss of crispiness. These samples had elasticity modulus values similar to those of commercially frozen spinach. Additionally, the isobaric sample 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. Notably, the mechanical properties of the isobaric sample immersed in the solution and the isobaric vacuum-packed sample did not significantly differ. Furthermore, these samples showed no significant changes in mechanical properties after 1 and 7 days of frozen storage (Bilbao-Sainz *et al.*, 2020).

- 3. Color Characteristics of Vegetables:** The color of fruits and vegetables is determined by natural pigments, some of which undergo changes as the plant matures and ripens. These pigments include fat-soluble chlorophylls (which create green hues) and carotenoids (responsible for yellow, orange, and red colors), as well as water-soluble pigments like anthocyanins (providing red and blue shades), flavonoids (imparting yellow hues), and betalains (producing red colors). In one study, researchers assessed how effective isochoric freezing is at preserving the quality of baby-leaf spinach. Initially, fresh baby-leaf spinach had the following color parameters: lightness (L^*) value of 43.9, greenness (a^*) value of 8.0, and yellowness (b^*) value of 20.7. The statistical analysis of color changes in frozen spinach leaves showed significant differences in the L^* parameter, indicating a darker color compared to fresh spinach. The greenness (a^*) values remained relatively consistent across all spinach samples, while the b^* values for samples treated with isochoric and isobaric methods in a solution decreased significantly after 7 days of freezing. Additionally, the spinach leaves had a somewhat translucent appearance, with isobaric and commercially frozen samples displaying more translucency compared to the isochoric sample (Bilbao-Sainz *et al.*, 2020).

One of the studies involved freezing potato cylinders within an isochoric system, various processing methods, freezing conditions, and compression rates were investigated. 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 maintained their yellow color regardless of freezing temperature, pressure, or compression rate. In contrast, vacuum-packed samples and those directly immersed in water showed some browning. Higher compression rates were associated with increased browning. Interestingly, there was a notable interaction between compression rate and the processing method. It indicated that, regardless of the compression rate, samples immersed in an AA solution retained their color, while vacuum-packed samples had higher browning index values compared to those immersed in water. Furthermore, samples immersed in water had significantly lower browning index values at the lower compression rate (Zhao *et al.*, 2021).

VII. IMPACT OF ISOCHORIC FREEZING ON MACROMOLECULES

It is crucial to carry out research on proteins at extremely low temperatures in order to increase storage efficacy, better transportation and improve lyophilization process. The preferred technique for obtaining subzero temperatures without freezing aqueous solutions is isochoric cooling, though it can speed up the cold-induced cleavage of polypeptides by

making them unwind. To hasten the formation of proteins, specifically the biological protein disulfide isomerase A1, isochoric chilling was employed. Osmotic techniques were used to dramatically improve the stability of protein isomerase A1 at -20°C , by using variety of solutions, such as sucrose, glycerol, and L-arginine. For instance, after 15 days of isochoric cooling at 20°C , the action of insulin had decreased by 22%. However, the breakdown of insulin was significantly curbed by a 0.6 M sucrose solution (Correia *et al.*, 2020). It has also been noted that isochoric cooling at subzero temperatures causes haemoglobin to aggregate, which is thought to have a substantial effect on reducing the impact of freezing stress (Rosa *et al.*, 2013). Additionally, the rate of haemoglobin aggregation increased exponentially as the temperature dropped, showing that subzero temperatures can encourage cold-mediated aggregation even in the absence of freezing stress.

In a different study, researchers investigated the relationship between temperature and pressure during the isochoric freezing of aqueous solutions that included glucose and glycerol, substances commonly used as cryoprotectants in traditional freezing methods. The study revealed that an increase in pressure during isochoric freezing can be harmful to biomolecules and can limit the temperature range within which isochoric systems can effectively be used for preservation, typically up to pressures below 40 MPa. However, by introducing glycerol into saline solutions at various concentrations, the researchers expanded the temperature range at which cryopreservation through isochoric freezing is possible. For instance, they achieved temperatures of -11°C with a 2 Molar solution of glycerol, -16.5°C with a 3 Molar glycerol solution, and -24.5°C with a 4 Molar solution of glycerol (Beşçea *et al.*, 2021).

VIII. ERADICATION OF MICROORGANISMS BY ISOCHORIC FREEZING

Generally, in microorganisms, the structure is modified during the process of isochoric 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.

IX. CONSEQUENCES OF ISOCHORIC FREEZING ON ENZYMES

It has been discovered that using isochoric freezing dramatically reduces food browning. For instance, potatoes held at $-5\text{ }^{\circ}\text{C}$ 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 $-4\text{ }^{\circ}\text{C}$ and $-7\text{ }^{\circ}\text{C}$ 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 $-3\text{ }^{\circ}\text{C}$ 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).

X. ISOCHORIC FREEZING FOR OTHER FOODS

This research investigates the potential of isochoric freezing for preserving chicken breast meat. The experiment entails submerging chicken breast samples in isochoric sodium chloride (NaCl) solutions at different concentrations (0%, 1.5%, and 2.5%) and temperatures ($-4\text{ }^{\circ}\text{C}$ and $-8\text{ }^{\circ}\text{C}$). The primary objective of this study is to evaluate how various process variables, such as temperature, pressure, and solution concentration, impact the quality characteristics of the chicken breast samples. These characteristics include colour, moisture retention, weight loss, texture, microstructure, and water mobility. The findings revealed that higher NaCl concentrations resulted in a reduction in pressure and temperature of freezing. Chicken breast samples subjected to treatment in pure water (PW) and a 1.5% NaCl solution at both $-4\text{ }^{\circ}\text{C}$ and $-8\text{ }^{\circ}\text{C}$ experienced a notable decrease in their quality attributes. Conversely, those treated in the 2.5% solution at $-4\text{ }^{\circ}\text{C}$ and $-8\text{ }^{\circ}\text{C}$ did not display any significant differences compared to the control group. These results indicate the possibility of improving the quality of preserved meats through the use of isochoric systems (Rinwi *et al.*, 2023). In terms of the

impact of various treatment conditions on loss of weight, there were significant reductions in weight loss. To elaborate, when freezing at -4°C , weight losses were 12.18%, 7.2%, and 1.5% as the NaCl solution concentrations increased from 0%, 1.5%, to 2.5%, respectively. Similarly, for isochoric freezing at -8°C , the weight losses were 14.17%, 9.62%, and 7.5% corresponding to NaCl solution concentrations of 0%, 1.5%, and 2.5%. The substantial weight loss observed in samples treated with pure water (PW) may be attributed to the formation of big ice crystals, resulting in structural destruction within the muscle bundle. This, in turn, resulted in drip loss and muscle fiber dehydration. In terms of color attributes, chicken samples treated with a 0% NaCl solution at both -4°C and -8°C showed significant increases in their lightness (L^*) and yellowness (b^*) values, while their redness (a^*) values decreased significantly. Conversely, chicken samples treated with a 1.5% NaCl solution at -4°C and those treated with a 2.5% NaCl solution at both -4°C and -8°C did not exhibit significant differences in L^* , b^* , and a^* values compared to the control group. As for texture characteristics, there were significant increases in hardness for chicken samples treated with increasing concentrations of NaCl solution (0%, 1.5%, and 2.5%). These hardness 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 , in comparison to the control with a hardness of 55.52 N. However, slight differences were observed in springiness, cohesiveness, and chewiness between samples treated with 1.5% and 2.5% NaCl solutions at both -4°C and -8°C . On the other hand, samples treated with pure water (PW) showed a significant increase in springiness, cohesiveness, and chewiness, with values varying depending on the freezing conditions. These observed changes in texture attributes can be attributed to underlying molecular processes, specifically protein denaturation, particularly involving myosin and actin. These proteins play critical roles in determining the texture of meat.

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).

Fish and fish-based products are of great importance in global nutrition and food security as they provide essential nutrients such as high-quality protein, omega-3 polyunsaturated fatty acids (PUFA), and vitamins (A, B, and D), as well as vital minerals like calcium, phosphorus, zinc, and iron (Bene *et al.*, 2016). This study aimed to investigate the impact of isochoric freezing on the freshness of tilapia fish in terms of attributes like color, texture, thiobarbituric acid reactive substances (TBARS), and total volatile basic nitrogen (TVB-N) content. It also sought to compare the outcomes of isochoric freezing ($-3^{\circ}\text{C}/37$ MPa) with other preservation methods, including chilling (5°C), super-chilling (-3°C), and freezing (-20°C). Under isochoric freezing conditions, the tilapia muscle experienced a slight increase in mass (less than 4%). Isochoric freezing also resulted in a minor yet significant rise

in water content, while salt content remained unchanged. In contrast, super-chilling and freezing led to mass loss due to drip loss. Fresh tilapia fillets had a slightly translucent appearance with a hint of reddish color. Isochoric freezing caused a notable increase in the L^* value and a modest but significant decrease in the a^* value, while the yellowness parameter b^* remained unchanged. Chilling didn't 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 increased both L^* and b^* values while reducing a^* values. Freezing produced color changes similar to those observed in isochoric freezing, and 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 had 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 maintained the structural integrity of muscle fibers by keeping osmolality comparable between extracellular and intracellular environments. This helped prevent cell dehydration and solute damage, a common occurrence in freezing at atmospheric pressure (Năstase *et al.*, 2017). In terms of textural properties, chilled, super-chilled, and frozen samples all exhibited decreases across all five measured properties. In contrast, isochoric-frozen fish displayed increased cohesiveness, indicating improved resistance to repeated deformations. Although all samples showed 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, which aligns with the findings from the micrographs of the muscle tissues (Bilbao-Sainz *et al.*, 2020).

The objective of the research was to investigate the fundamental processes governing 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 explored how these treatments, carried out at -4°C using a 2.5 g/dL sodium chloride solution, affected the overall quality of the chicken breast meat. The study unveiled that various moisture transfer mechanisms, including diffusion and infusion, played a crucial role in altering the distribution and mobility of water within the meat. These changes had significant and quantifiable 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 showed only slight effects, with noticeable differences primarily in pH and WHC. VPF-treated samples, on the other hand, did not exhibit any significant deviations from the characteristics of fresh samples. In conclusion, this study suggests that isochoric freezing protocols can be customized to suit specific sample types and desired outcomes, offering valuable theoretical and practical insights for the development of tailored isochoric freezing protocols (Rinwi *et al.*, 2023).

XI. 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 $-5\text{ }^{\circ}\text{C}$ 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.

XII. 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.

XIII. 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.

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