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IMPROVING THE FREEZING RESISTANCE OF BAKER'S YEAST. A MINI REVIEW

Ioana ISACHE[1], Adriana DABIJA [1], Amelia BUCULEI [1], Ancuța CHETRARIU[1]*

[1]*Faculty of Food Engineering*, Stefan cel Mare University of Suceava, Romania, 720229

*Corresponding author: ancuta.chetrariu@fia.usv.ro

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Abstract: *Frozen dough technology is widely used and may guarantee bread's freshness and so prolong its shelf life. However, a variety of issues, including the restriction of yeast activity and structural damage to the dough, may arise while frozen dough is being made and store, ultimately resulting in a loss of quality. After fermentation, the bread's ability to retain CO_2 and the yeast's ability to produce CO_2 determine the frozen dough's quality. Ice crystals are thought to be the primary cause of both decreased viability of yeast and the breakdown of the dough network structure, which two significant elements are contributing to the decline in dough quality. Several factors affect yeast's resistance to freezing and thawing, such as its physiological state before freezing; for instance, yeast cells in growth standstill are more resistant to freezing than those in the exponential growth phase. Numerous strategies and methods have been created to raise the freezing baker's yeast resistance and, consequently, the quality of frozen dough. These strategies include the use of genetic engineering, the incorporation of chemicals like hydrocolloids and antifreeze proteins (AFP), the improvement of freezing times and circumstances of storage, and the creation of novel freezing techniques like ultrasonic freezing. These techniques for enhancing the freezing resistance of baker's yeast are outlined in the publication.*

Keywords*: antifreeze proteins, bakery products, freeze-tolerant yeast strains, ice nucleation, shelf life*

1. Introduction

Cereal and bread product production has been continuously changing due to consumer expectations, consumption patterns, and the desire of bakeries to reduce labour costs [1]. At first, the industrialization of the bread-making process resulted in significant savings in terms of time and money. But the bread market is the least adaptable to the new shifts in society, the consumption of bread decreased steadily until the mid-1990s. This decline was partly caused by bakery goods' short shelf life, since their quality primarily depends on how long they are baked before being consumed. Bread aging results in a loss of consumer acceptance [2, 4].

Grain-based bakery goods were a significant source of nutrition for people [5,6]. For instance, bread has become a staple of the diet due to its rising consumption over time. For the year 2021 alone, this industry has accumulated

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approximately USD 117.65 billion in revenue globally [6].

A revolutionary change began when, in the bread market, low temperatures were appliedallowed night work in this industry to be eliminated. First strategy involved freezing store-bought bread, but successfully keeping bread fresh depended heavily on proper control of freezing and thawing rates, proper storage conditions, and using only fresh bread. But frozen bread could not be stored for longer than a few days without losing its fresh baked quality [7, 8]. By using this tactic, the fermentation process was delayed when making refrigerated dough. However, the shelf life of refrigerated dough was very short and long-distance distribution was not possible [9, 10].

Although freezing has long been used in this industry, since the 1980s, a small market has evolved into a large-scale enterprise. Since almost everyone consumes bread, the market for bread was thought to be well-established and to have nearly complete penetration; nonetheless, there were certain niches within this market that had significant room for expansion. A number of theories have been proposed to account for the bakery industry's phenomenal rise, such as the variety of products that keep consumers interested in the bread market, the use of ingredients that are currently considered healthy, the addition of complex flavors and textures that improve the bread's appeal, and the expansion of distribution channels. The use of low temperatures in bakeries provides a simple method for processing fresh, chilled, and frozen bread, ensuring a steady rate of expansion in this industry [2].

Research undertaken on bread and dough freezing followed a parallel trend with market importance. The range of goods that can be obtained after fermentation and baking in the so-called hot areas has led to a tremendous expansion in the production of frozen dough in recent decades [11, 12]. From the perspective of the baker, there are financial benefits because frozen dough production does not require highly skilled workers. Large organizations use automated procedures to obtain frozen dough, which can save production costs and provide a consistent quality output from the producers. However, the process of making bread now has additional needs in terms of raw materials, machinery, packing, and transportation due to the creation of frozen dough [13, 14].

2. Technological process of obtaining frozen doughs. Process parameters and influence on yeast viability

The technological process of obtaining frozen products involves kneading the raw materials, shaping, leavening, and baking. The introduction of freezing technology includes new steps such as packaging after moulding, freezing, frozen storage, thawing, leavening, and baking. Bakery dough begins to freeze when it comes into contact with low temperatures, usually below -18 °C. It is dependent on the rate at which ice crystals form; if large crystals grow disorderly, they will damage the product's structure; if small crystals form, their growth will not affect the integrity of the cell wall. Additionally, the water absorption index of wheat flour starch should be taken into account while creating a frozen dough, as this establishes its ability to hold onto water and its impact on the matrix's texture [15]. According to Kondakci et al. [16] foods go through multiple stages of freezing, including pre-cooling, nucleation, and tempering [16]. During the pre-cooling phase, the food's temperature is progressively lowered from its starting position to the freezing point without causing any physical alterations to the matrix. Next comes nucleation, a process that raises the temperature to its stable freezing point as the food matrix uses

the available water to spontaneously form ice nuclei in its structure. Eventually, throughout the freezing and storage phases of tempering, the matrix stabilizes and eventually reaches the desired temperature [17]. In the baking sector, kneading the components is the method used to generate frozen dough. This is evident in the standardization of the fresh product production process, after which portions are formed in accordance with predetermined specifications. After that, the dough is put in a freezing system with low temperatures until the interior temperature reaches -18 °C in the center. The dough then completes its frozen state by the interchange of the matrix's specific heat. It is possible to change when a baked good should be frozen, such as after the kneading stage, after the dough has been proofed, or after the product has partially baked. Additionally, the formation of intracellular ice changes the structure of the product and may therefore limit the yeast's ability to survive [18]. The methods of manufacturing bakery products from frozen dough are very diverse, and they differ essentially by the moment of application of freezing, the temperature and speed of freezing, the temperature of the dough before freezing, the duration of storage in the frozen state, the use of improvers baking, the addition of non-traditional foodstuffs, the use of resistant yeast strains [19]. It has been demonstrated that advancements in frozen dough baking product technology, the main disadvantage is the reduction of the sensory properties of the finished product (outer appearance, volume, and porosity) compared to products obtained from non-frozen dough, it is linked to the gluten network's weakening and is in charge of fermentation gasses' retention; another disadvantage is it a partial destruction of yeast cells, accompanied by a reduction in the amount of gases removed in the fermentation process after thawing. Depending on the flour's quality, the final product's sensory qualities deteriorate to varying degrees, the quantity and quality of the yeast used in the manufacture of bakery products, the recipe of the product and the addition of additives, the conditions of kneading and fermentation of the dough, the temperature and speed of freezing the dough, the duration of storage in the state frozen and the thawing method applied [12]. Nonetheless, phenomena that take place during freezing and storage in the frozen state are connected to the decrease in the volume of frozen dough. such as the decrease in the fermentative capacity of the yeast and the loss of the integrity within the gluten network [20]. This behavior affects the workability of the dough and creates a problem in the industrial chain (reduction of the dough's shelf life) due to the decreased quality of the dough (losses of strength during fermentation). The dough's gluten network breaks as a result of the strength decline, which causes poor gas retention and volume loss when baking [21]. These occurrences most likely result from the water in the dough matrix being unevenly redistributed after freezing. On the other hand, studies have shown that variations in temperature while storing and transport have altered the quality of frozen dough due to recrystallization of crystalline ice. In fact, the rate at which carbon dioxide is formed varies on the number of yeast cells, the strain, the yeast's physiological condition and the quantity of fermentable sugars available [22]. The viability and activity of the yeast cell are impacted by both freezing and frozen storage. Since carbon dioxide is mostly produced by yeast, the amount produced was used as a measure of yeast activity. The impact of freezing on yeast characteristics has been documented in a number of studies, which is a significant problem when producing frozen dough. Studies have shown that there is a major influence on yeast viability by the time and

temperature of the dough before freezing [23, 25]. When dealing with frozen dough, it's critical that the dough's temperature prior to freezing stays below 20 °C in order to stop fermentation from starting. This behavior can be explained by the yeast cells' extreme sensitivity to osmotic pressure-induced damage within the dough matrix [26]. Since freezing speed controls yeast activity, it can be regarded as a critical determinant of frozen dough quality. The freezing rate needs to be both quick enough to limit the effects of solution concentration induced by water crystallization and slow enough to prevent the development of ice crystals inside the cell. The harm that high concentrations and temperatures can do to yeast membranes has been covered by other writers [27]. However, by adopting the modified manufacturing method and taking the necessary precautions during formulation, freezing and storage injuries can be reduced to a minimum. The amount of yeast utilized in frozen doughs varies depending on how long the dough is left to ferment after thawing, how long the dough is frozen, and how the dough is made. Certain technological parameters, including freezing temperature and speed, should be taken into account to enhance freezing performance. Additionally, the type of yeast used and the amount of yeast added to offset the loss of fermentation activity should be taken into account. This procedure entails adding, based on the length of frozen storage, 50-100% more yeast than the recommended amount. On the other hand, adding too much yeast might result in a yeasty taste in the finished product and raise production costs. The impact of frozen storage duration on the textural and rheological characteristics of dough has been documented by a number of writers. The mechanical action of ice crystals during freezing and storage causes structural changes in the frozen dough as well, which deteriorates the frozen dough network and results in decreased dough strength and inadequate gas retention during fermentation [28].

Furthermore, the release of reducing agents by the yeast, including glutathione, is linked to the degradation of the gluten network in frozen dough [5, 29, 30].

3. Strategies and methods to improve the behaviour of baking yeast in dough that is frozen

3.1. Food additives usage

In the bakery sector, freezing technology can have an impact on the frozen dough products' quality. Cryoprotective chemicals are recommended in order to partially prevent ice nucleation and recrystallization during subfreezing temperature storage, hence enhancing the end product's quality [31, 32].

Each additive should have an ideal dosage because the effects of additives on frozen dough vary depending on their quantity, formulation, and processing circumstances. Typical ingredients for frozen dough include hydrocolloids, antifreeze proteins (AFPs), ice nucleating agents, and other dough conditioners [6, 33]. The effects of different additives on frozen dough are presented in Table 1.

3.1.1. Emulsifiers

The majority of the research done thus far has focused on the addition of lipids associated with emulsifiers, such as sucrose esters and diglycerides. Emulsifier addition resulted in the avoidance of starch retrogradation because of the interaction of starch with emulsifiers and the reduction of moisture migration between gluten and starch [34].

3.1.2. Antifreeze proteins (AFPs)

Currently studies are focused on the use of innovative additives like ice protein nucleating agents (AFPs) and ice

structuring proteins (ISPs). AFPs, also called ISPs, are made up of a series of proteins that can improve the body's frost resistance [8].

Table 1.

Effects of different additives on frozen dough

Cryoprotectant category	**Additives**	**Mechanism of action**	**Results**
Emulsifiers	Emulsifiers	inhibits the movement of moisture from the dough network to the outside, which aids in lowering the surface tension of gas bubbles by interacting with starch to limit water absorption.	starch retrogradation delayed as a result of freezing
Antifreeze proteins (AFPs)	Antifreeze proteins (AFPs)	The ability of AFPs to modify crystal shape, impede ice recrystallization, and exhibit thermal hysteresis capacity (THC) are the basis for their characteristics.	enhanced gluten network; slower rate of stiffening
Ice-nucleating agents	Ice-nucleating agents	Although the adsorption-inhibition theory underpins the mechanism of action of AFPs, the precise function of hydrogen and hydrophobic bonds/residues as well as structural features are also thoroughly explained.	reduced damage to glutenin macropolymers, changed the form of ice crystals, and prevented ice from recrystallizing.
Hydrocolloids	Locust Bean Gum Guma Tragacanth Xanthan gum HPMC	Minimize the harm that ice crystals cause to breads made from frozen dough or partially baked frozen bread. Hydrocolloids bond with water, create hydrophilic complexes with gluten proteins, and stop moisture from migrating through the dough.	Preventing water crystallization and preserving frozen dough's rheological characteristics
Wheat floury materials	Whole wheat flour Waxy wheat flour	Boost the capacity to hold water and stop it from migrating and spreading.	lowering the amount of freezable water and enhancing dough quality by raising the dough's capacity to absorb water and decreasing its stickiness

In their studies, Zhang et al. [19] introduced the leucine-rich protein Daucus carota antigen protein (DcAFP) to frozen dough and found that DcAFP could protect yeast from freezing. cell membrane damage upon freezing, controlling ice crystal distribution, and inhibiting crystal formation seas of ice [19]. The thermal characteristics of frozen dough were considerably impacted by the addition of winter wheat ISPs., such as lowering the freezing point peak, reducing the freezable water content, decreasing the effective thermal conductivity and lowering the specific heat peak in the dough frozen [6, 33]. Proteins with antifreeze properties were first identified in fish but have also been discovered in other creatures like insects, plants, and microbes that live in environments with cold temperatures.. These proteins have been classified as type I, II, or III antifreeze glycoproteins (AFGPs) or AFPs depending on their structural features. The functions of all antifreeze proteins that have been found thus far are identical, despite this structural variation. They prevent ice from recrystallizing and, at comparatively low concentrations, reduce the freezing point of water without changing its melting point (thermal hysteresis). Antifreeze proteins bind directly to the ice surface via hydrophobic and van der Waals interactions. In this way, even at very low protein concentrations, they prevent water molecules from continuing to bind and modify the structure of ice crystals. In addition, they have been attributed the function of protecting the cell membrane at low temperatures [6].

3.1.3. Ice-nucleating agents

The production of ice and its regulation by ice-regulating materials have drawn a lot of attention in recent years [35]. A distinct interface within the mother phase is formed during the crucial nucleation step by direct assembly of monomers driven by density fluctuations over the bulk free energy barrier and surface. Depending on the foreign contact, nucleation can happen through either homogeneous or heterogeneous nucleation, driven by supercooling (ΔT) [36]. Ice recrystallization is brought on by the instability of newly formed ice crystals, which includes differences in their number, shape, size, orientation, and perfection. (Figure 1). In extremely low temperatures, heterogeneous ice nucleation can be triggered by ice nucleating proteins (INPs). INPs are typically found in a wide variety of gram-negative, epiphytic, pathogenic ice nucleation active (INA) bacteria [37, 38].

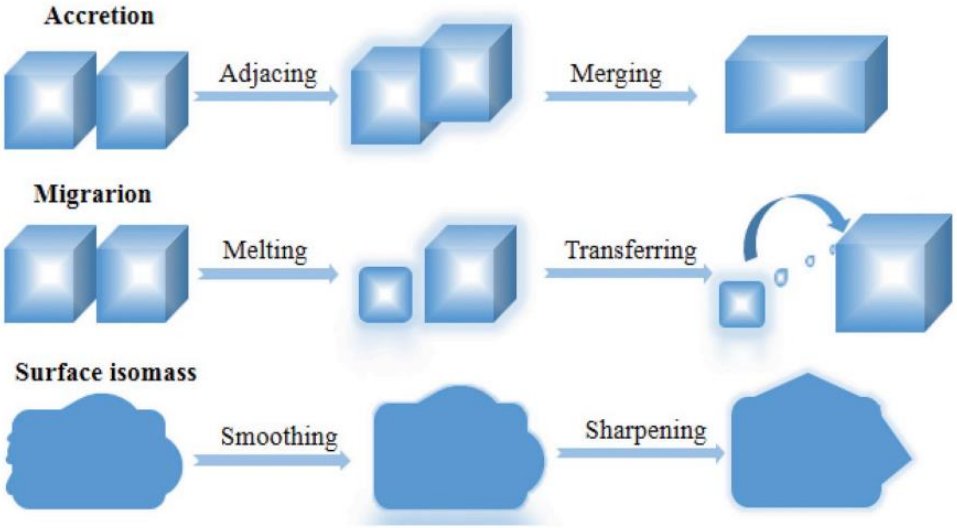

Fig 1. Three mechanisms of recrystallization (accretion, migratory, and surface isomass) [37]

Heterogeneous ice nucleation, which happens at higher temperatures, is the main mechanism for ice production during the freezing and frozen storage of dough. Large ice crystal formation is inhibited and the degree of supercooling is primarily minimized by ice nucleating chemicals. *Pseudomonas syringae* and *Erwinia herbicola*, two common epiphytic bacteria found in plant leaves, have been the subject of much research because they prevent plant supercooling, which is what causes the ice to form [39]. Adding *Erwinia herbicola* extracellular ice nucleators to frozen dough decreased bread crust's firmness and increased specific volume by 50% after 3 freeze-thaw cycles compared to the control sample[40, 41]. The nucleation temperature and chemical composition of microbial ice nucleating agents can be used to classify them. The amount of lipid, protein, saccharide, and polyamine that *Erwinia uredovora* produces in extracellular ice nucleators was reported to be 10%, 43%, 35%, and 12%, respectively, in a prior study. This confirms their lipoglycoprotein nature. Zein-based extracellular ice nucleators (INFs) have been used to protect frozen dough. After five freeze-thaw cycles, the temperature at which water begins to form ice increased from -15 to -6.7 °C, and the amount of water lost through frozen storage decreased. When compared to the control dough, the specific volume of the bread increased by 25%. This is explained by the yeast's higher vitality due to cryopreservation [40].

The primary purpose of intracellular ice nucleating agents is to reduce the amount of supercooling and prevent the production of big ice crystals. The primary constituents of extracellular ice nucleators (ECINs) derived from *Erwinia herbicola* are proteins, polysaccharides, polyamines, and lipids. Because the extracellular ice nucleators prevented the yeast cells from being damaged during freezing, the bread produced was on par with fresh bread. The bread made with frozen dough had less volume than fresh bread, while the bread produced with ECIN added was of greater quality. Furthermore, the bread with extracellular ice nucleators added had a crust color that resembled fresh bread. Additionally, according to Luo et al. [42], zein-based ice nucleation films (INF) would raise the ice nucleation temperature from -15 to -6.7 °C, decreasing the amount of

moisture losses and the detrimental effects of the freeze-thaw cycle on the dough [42].

3.1.4. Hydrocolloids

By adding hydrocolloids, freeze-thaw cycles can have a less detrimental effect on the frozen dough, preserving its rheological characteristics. Large ice crystals may form during storage at -18 ℃ due to the recrystallization of free water. It functions as a hydrophilic colloid and can create hydrophilic complexes with binding water and gluten-forming proteins. Consequently, adding hydrocolloids can help the dough retain more water, which will lessen the amount of liquid that seeps into it. Furthermore, because hydrocolloids can bind water to their structure, they can reduce water activity [43, 44].

Numerous studies have been conducted on hydrocolloids, which are additives used to regulate the rheology and texture of aqueous food systems. The instability of frozen dough qualities during freezing storage is associated with temperature fluctuations and ice recrystallization. Frozen dough can be preserved by hydrocolloid by complexation with bound water and gluten. This reduces gluten damage from ice recrystallization in the frozen dough, modifies moisture migration, and raises the dough's water holding capacity (WHC). Because hydrocolloids compete with other wheat ingredients, they also reduce water activity. Depending on the type of hydrocolloid utilized, different hydrocolloids interact differently with the dough's macrocomponents (water, starch, and proteins). For instance, the addition of pectin or xanthan gum resulted in increased and decreased molecular mobility of the gluten-water matrix, respectively. Consequently, the sort, dosage, solubility, water-holding capacity, rheological properties, and their synergistic interaction with other components during freezing and frozen storage all affect how successful hydrocolloids are as a cryopreservative overall in frozen dough products [8]. Some of the most often used hydrocolloids in the baking industry are carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC), guar gum, xanthan gum, carrageenan, and others. Because hydrocolloids are able to interact with both the water network and gluten, they can hold more water, which changes the moisture content and lessens the harm that ice crystal formation causes to the gluten. The bread may bake more quickly as a result of this decrease in ice crystal size, which may aid in the development of the crust and core structures and assist the dough matrix retain its organoleptic qualities. In comparison to the non-frozen dough, the fermentation rate significantly dropped in all treatments following the storage period and thawing cycle. It suggested that the yeast cells' ability to create and sustain the gas inside the structure was limited by the freezing process. It is crucial to keep in mind that a variety of factors, including the quantity added, solubility, water retention capacity, impact on food component interaction and storage duration, and the rheological characteristics of the gluten matrix, can all affect how effective adding inhibitors is [18, 45].

Gums and other hydrocolloids are frequently found in frozen pastry dough for baked items. They are able to stabilize the moisture content by shielding the structure during the nucleation stage because of their contact with the gluten network. One benefit is that the dosage has been lowered to a maximum of 1% to guarantee its efficacy. It's also critical to take the matrix's rheological characteristics, moisture-holding capacity, and solubility into account. As a result, the industry can use these changes as a technological challenge to enhance frozen baked items' texture and lengthen their shelf life. Sim et al. [46] revealed in their studies that the addition of

carob seed gum *Ceratonia siliqua* potentially change the melting characteristics of frozen dough and prevent free water from recrystallizing. due to its high-water absorption capacity and better sensory quality [46].

3.1.5. Wheat flour

Whole wheat flour and waxy wheat flour are two examples of floury raw materials that can be added as additives to enhance the frozen dough's rheological characteristics. According to reports, adding whole wheat flour that is high in fibre to frozen dough may result in strong hydration and better plastic characteristics, which could enhance bake ability [46, 49].
According to Jia et al. [50], adding 10% waxy wheat flour to ordinary flour could raise the temperature at which gelatinization occurs, limit molecular water movement and redistribution, and considerably lower the amount of damaged starch in frozen dough [50].

3.1.6. Baking enzymes

The manufacturing process of frozen fermented dough incorporates a number of ingredients (emulsifiers, enzymes, and oxidants) that can improve the characteristics of the finished products, such as the volume, crust, crust, and texture of the bakery products. The big problem in using conventional commercial enzymes (amylase, gluco-amylase, glucose oxidase, xylanase, lipase, etc.) to improve frozen dough is that the enzymes stop working during storage (low molecular kinetics). When the dough is thawed at room temperature and before baking, there is not enough time for the oxidizing action of gluten, even in the case of an overdose of enzymes, starch hydrolysis products as a nutrient source for yeast and the formation of amino compounds in the Maillard reaction which should provide colour, necessary for baked products. The result is a product of lower quality compared to a freshly produced and non-frozen product, reduced volume, reduced colour, and skin more prone to aging, a phenomenon caused by starch degradation [31].
There is a new generation of cryoprotectants that can prevent yeast (*Saccharomyces cerevisiae*) lysis during rapid freezing and ice crystal formation, including the use of cryoenzymes or enzymes derived from microorganisms capable of remaining active and stable under extreme conditions. When compared to their thermostable counterparts, cryoenzymes-enzymes derived from cryophilic microorganisms-have the ability to function at negative temperatures and have developed a number of structural traits with a high degree of protein flexibility. Low activation enthalpy, strong substrate affinity, and high specific activity at low temperatures are all a result of the great flexibility, particularly in the vicinity of the active site. All aspects lost during the freezing process of sourdough products can be improved with the usage of this class of proteins. When bread is stored, its sensory quality usually deteriorates., such as feeling dry, an increase in firmness of the core, loss of flavour, and softening of the crust. Dough loosening during frozen storage and a decrease in yeast activity and viability have a negative impact on baked frozen dough quality. Dough stored in the freezer affects the gluten network, reduces its ability to hold gas, and lengthens the fermentation process. After 60 days of storage at -18 °C, the gluten matrix in dough might be seriously degraded, resulting in holes appearing in the gluten micelles [51]. Frozen storage also led to certain issues with bread quality, including poor volume and texture deterioration, which were brought on by a number of variables., including reduced gluten cross-linking, ice crystallization and recrystallization,

redistribution of water and the release of reducing substances from the yeast [52].

Proteins called enzymes facilitate chemical reactions and are therefore employed as additions in a variety of flour and bakery goods, including bread, cakes, biscuits, and the like. Research has shown that certain enzymes can enhance the sensory qualities of bread by enhancing the flour's quality, which results in a dough that is easy to work with, and by enhancing the bread's structure. To enhance the flour's fermentation capabilities, fungus-derived α-amylase is incorporated into the dough. Some of the starch is broken down by them into simpler substances like glucose, maltose, and dextrin. This leads to an improvement in the crust's flexibility and an increase in the volume of a baked good. Dextrin stops gluten and gelatinized starch granules from forming cross-links and from further breaking down amylopectin. This helps to delay the aging process and maintain the bread's freshness for an extended amount of time. Water-insoluble arabinoxylans are hydrolyzed into smaller water-soluble components by xylanase, also referred to as pentosanase. These soluble in water substances raise dough viscosity and stabilize gas cells. Xylanase increases gluten resistance by redistributing water between the pentosan phase and gluten. As a result, the dough's flexibility improves during baking, increasing the product's volume. When xylanases are used, big, water-insoluble pentosan particles are broken down into smaller, soluble particles. This, in turn, contributes to the inhibition of the aging process of bakery products [31].

In the presence of oxygen, glucosease catalyzes the conversion of α-D-glucose to α-D-gluconolactone, producing H_2O_2. This, in turn, oxidizes hydrogen sulfide (-SH), increasing the tensile and elastic properties of dough [53]. Glucose oxidase does have certain drawbacks, though. For example, during the first stage of dough development, it oxidizes quickly, releasing H_2O_2 quickly and causing the dough to overrise and ultimately have a low volume. Glucosoxidase increases the specific volume of baked goods by strengthening gluten, catalyzing the oxidation of glucose, and increasing CO_2 generation. It strengthens the core's structure and mitigates the effects of dough freezing, which promotes the formation of more protein links. The bakery product's crust gets more cohesive and elastic in this way [53]. Lipase transforms triglycerides into free fatty acids, glycerol, and either mono- or di-glycerides. The mono- and diglycerides are liberated from the lipids by the additional lipases, which improves the volume and suppleness of the bread and helps postpone the aging process. The hydrolysis of 1,4-glucosidic bonds in α-D-glucose residues is catalyzed by amyloglucosidase. This process commences with the non-reducing ends of maltooligosaccharides and polysaccharides, and ultimately releases β-D-glucose. Most versions of the enzyme may hydrolyze rapidly. 1,6-α-D glycosidic bonds occur when 1,4-glycosidic bonds follow in order. This accelerates the process of fermentation. Consequently, this has a favorable impact on the bread product's volume and stability throughout freezing.

Transglutaminase (TG) can promote the inter- or intramolecular crosslinking of glutamine and lysine residues to form high-molecular-weight gluten. TG can strengthen the quality of whole wheat dough by improving its protein network during frozen storage, making the frozen dough products less firm [54,55]. In a different study, transglutaminase treatment significantly enhanced the ratio of high-molecular-weight gluten subunits and reduced the viscoelasticity loss that results in frozen dough. Table 2 shows the mechanism of action of enzymes and effects

of different enzymes on frozen dough quality.

3.1.7. Other additives

Polyols, sometimes referred to as sugar alcohols or sugar replacements, are low-sweetening carbohydrates classified into monosaccharides such as erythritol, mannitol, sorbitol, and xylitol and polysaccharides such as isomaltitol, lactitol, maltitol, and trehalose [56, 57]. These compounds are found naturally in fruits, but industrially, they are made from other carbohydrates such as starch, sucrose, and glucose. In addition, they have a lower caloric content than sucrose, because they are not fully absorbed in the small intestine, their glycemic response is lowered. In baked goods, where the impacts of low temperatures impair the final product's quality, the use of these polyalcohol's can lessen the consequences of dough freezing.

Table 2.

Effects of different enzymes on frozen dough quality

Enzyme	**Mechanism of action**	**Results**	**Final product quality**
Xylanase	Breaks down larger, water-soluble arabinoxylans by hydrolysis.	Boost dough viscosity and stabilize gas cellsImproves gluten resistance	Increased volume Inhibition of the aging process of bakery products
Glucosoxidase	Catalyzes the oxidation of hydrogen sulfide (-SH) to H_2O_2 by converting α-D glucose to α-D gluconolactone in the presence of oxygen. serves as a catalyst for glucose oxidation.	Increase dough tenacity and elasticity Strengthens gluten and enhances CO_2 production	Increased specific volume Crust of the bakery product becomes more elastic and more cohesive.
Lipase	Converts triglycerides into free fatty acids, glycerol, and mono- or di-glycerides via catalysis.	The fats release the mono- and diglycerides.	Improved bread softness and volume
Amyloglucosidase	By hydrolyzing the 1,4-glucosidic linkages in α-D glucose residues, starting with the non-reducing ends of polysaccharides and maltooligosaccharides in a stepwise manner, it catalyzes the release of β-D glucose.	Fermentation process is accelerated	Improved volume and stability during freezing
Transglutaminase	Can encourage the formation of high-molecular-weight gluten by the intra- or intermolecular crosslinking of lysine and glutamine residues.	Enhances the protein network while being frozen	Less firm frozen dough products

Trehalose can improve the behaviour of the dough under freezing conditions leading to an improvement in the final product's quality in terms of its texture and bread volume. Bread structure was shown to be shielded by trehalose against the development of ice crystals during freezing and thawing, which improved bread texture. Trehalose also reduces water mobility in dough and bread, resulting in an improvement in bread freshness [31, 44, 52, 58, 64]. When ice crystals form during the freezing process, water migrates and moves around, causing redistribution of water in goods created from frozen dough. Polyols with a high water-holding capacity, such sorbitol and mannitol, facilitate the development of gel during the dough-making process due to their hydration behaviour. By raising the volume and caramelizing the crust, this behaviour helps to strengthen the gluten-starch network that has formed, giving the dough a better texture and crunch. Like trehalose, glycerol exhibits cryoprotective properties. The enhanced ability of the dough to leaven, the shorter freezing time following the initial freeze and thaw, and baker's yeast's enhanced ability to withstand freeze-thaw stress after glycerol was added all suggest the possible application of intracellular glycerol-enriched cells in frozen dough.

3.2. Use of freeze-tolerant yeast strains

It has long been believed that the use of freeze-tolerant yeast strains goes against the principles of biological design. The reduction in fermentative activity brought on by freezing resistance has not been entirely remedied by either physiological conditioning or the selection of strains resistant to freezing temperatures. Therefore, the idea that high fermentation capacity and robust stress resistance could be incompatible biological qualities [2, 65]. The creation of yeast strains that are more resistant to freezing, particularly during active fermentation, might offer a more workable solution to the issues associated with producing frozen dough. Through mutagenesis and surveys of naturally existing isolates, freeze-tolerant strains of yeast have been identified. But no strains exist that combine the best commercial strains' ideal pleiotropic phenotype with increased freeze tolerance [66, 67].

The freeze-thaw in *S. cerevisiae.* The integration of the stress response with a broader network of stress responses, including as oxidative stress, shock from cold and heat, and others, makes it significant in part. Certain genes and processes, such as the cell wall integrity (CWI) pathway, proteasomal activity, and nutrient sensing signaling, are associated with the ability to withstand other types of stress and provide freeze-thaw resistance. Biochemical protectants, such as lipid components that provide membrane flexibility and metabolites that hinder ice crystal formation and facilitate the scavenging of reactive oxygen species (ROS), can be used to broadly classify other resistance factors. As research advances beyond *S. cerevisiae* alone, comparisons to cold-adapted organisms have offered significant insights into a range of diversification in yeast freeze-thaw stress responses [68, 69].

For freezing stress there is no true response, therefore survival depends mainly on the cell's physiological condition prior to freezing. After several freeze-thaw cycles, either with or without a recovery interval in between, no cellular adaptation was seen, suggesting that yeast cells are not able to withstand the freezing stress brought on by frequent freezing and thawing. It is less likely that yeast cells will be able to manufacture or change stress proteins or stress metabolites when they are frozen since they have a very limited amount of time to adjust to this new environment. An improvement over freezing It has been

reported that heat treatment prior to freezing increases the tolerance of commercial baker's yeasts in dough [70, 73]. However, industrially, the faster loss of stress resistance brought on by heat-induced fermentation stimulation appears to offset this impact [74].

In the baker's yeast industry, where molasses is used as a substrate and fed-batch production is the commercial method, it is recognized that altering the growth circumstances can modify the stress tolerance of yeast cells. The feeding parameters, aeration conditions, and vessel size all affect the successive propagation processes that make up the fed-batch fermentation method. It has been demonstrated that fed-batch cultured cells, particularly those grown at slower rates and with robust aeration, are more resistant to freezing than cells grown in any batch culture phase [66, 72, 75]. Trehalose cell storage can increase the freezing tolerance of yeast cells. In freeze-thaw stress, physical factors such ice crystal formation and dehydration are mostly to blame for the majority of the cell damage, which occurs more during the freezing phase than during the thawing phase. Trehalose is a non-reducing disaccharide that is characteristic of yeast cells that can quickly adjust to their surroundings. It serves as a stress reliever and a store of carbs. Trehalose stabilizes cell membranes to act as a thermoprotectant and cryoprotectant. It builds up dramatically in cells subjected to non-lethal heat shock [71, 73]. Trehalose's propensity to absorb water, which is a result of its hydroxyl groups, which give it hydrophilic qualities, underpins its mechanism of action. In addition, decreased water diffusivity lowers the freezing point and protects yeast membranes from freezing damage, osmotic dehydration, and denaturation [76]. Throughout their life cycle, *S. cerevisiae* cells undergo significant variations in their trehalose content and level of stress tolerance in response to their surroundings. In yeast cells that are developing exponentially, the amount of trehalose is relatively low; it increases when the cells enter stationary phase or are exposed to stressors. Trehalose accumulation seems to occur during the stationary phase of glucose growth as well as during times of decreased growth, such as when cells are deprived of nitrogen, phosphate, or sulphur [44]. Trehalose buildup strengthens yeast's tolerance to stress when it grows on non-fermentable carbon sources [44]. Trehalose also acts as a barrier against dehydration, allowing the membrane to remain intact by dislodging water molecules and attaching them to phospholipid polar groups. Moreover, trehalose guards against H_2O_2 damage to biological proteins and yeast cells., reduces the intracellular concentration of reactive oxygen species, and decreases lipid oxidation in vivo during menadione exposure [77].

Trehalose level in the yeast *Saccharomyces cerevisiae* can vary from less than 1 to more than 20% of the dry weight of the cell in response to stress or famine. Cells that are experiencing carbon and energy limitation both during stationary phase approach in a rich medium and during diauxic growth in a comparatively poor medium acquire trehalose. The first exponential phase of growth is devoid of trehalose production. However, with a modest stress, like a heat treatment at 37-40 °C, yeast cells that grow exponentially on glucose can accumulate enormous levels of trehalose. With the end of stress or when nutrient intake is replenished, this sugar breaks down quickly. Imbalance between synthesis and hydrolysis enzyme activities controls intracellular levels of trehalosein in *S. cerevisiae*. Trehalase is necessary for trehalose cleavage. The species *S. cerevisiae* has three different trehalases: Nth1p, Nth2p and Ath1p [77, 79].

Many yeast species, besides *S. cerevisiae*, also manufacture trehalose through the trehalose-6-phosphate synthase (TSP)/trehalose-6-phosphate phosphatase (TPP) pathway. However, *S. cerevisiae* stands out for having a TPS complex with four subunits, two of which are regulatory and the other two of which are enzymes. This suggests that the trehalose production in this yeast is more complexly regulated. Trehalose-6-phosphate, a trehalose metabolic step, may have a regulatory effect on glycolysis and fermentation, which explains why *S. cerevisiae* uses a protein complex to manufacture trehalose. Another hypothesis is that additional trehalose-producing species do not yet have regulatory subunits discovered. In addition to Tps1p and Tps2p, Tsl1p was originally believed to be a part of the TPS complex. Later on, it was found that the fourth subunit was Tps3p, a homolog of Tsl1p. Tsl1p and Tps3p interact with Tps1p and Tps2p, who in turn interact with each other, rather than with each other, as demonstrated by a two-hybrid technique [61].

Although current production conditions allow obtaining stress-tolerant yeast including freezing stress. The fundamental issue with frozen dough technology, which is the decreased viability of yeast cells when they come into contact with the nutrients in the flour, is not resolved by this. Consequently, it appears that physiological conditioning cannot address this core issue.

3.2.1. Strain selection and isolation

Not only have freeze-tolerant yeast strains been isolated from natural sources such as soil, cereals, and fruits, but they have also been selected from collections of strains and traditional corn and rye bread doughs. [72]. While *S. cerevisiae* strains are frequently utilized in bread production, research has also been published on strains of *Torulaspora delbrueckii* and *Torulaspora pretoriensis*, which typically exhibit greater resistance to frost than traditional baking yeast strains but are frequently hampered by maltose fermentability [80]. Generally speaking, traditional breeding methods have not been sufficient to generate industrial strains fit for use in frozen dough. One of the main issues with changing basic yeast traits, like stress tolerance, is that other desired traits would almost certainly change as well. This occurs so frequently that, for instance, great stress resistance was previously believed to be incompatible with rapid growth and fermentation [72]. Table 3 highlights the important biological criteria in selecting yeast strain with high freezing resistance.

Table 3.

Important biological criteria in selecting yeast strain with high freezing resistance

Parameter	**Criteria**
Yeast strain	Hybrid yeast Recombinant cryotolerant yeast Osmotolerant yeast
Yeast composition	Intracellular glycerol The ratio between phospholipids and free sterols Protein content Factors that increase the accumulation of trehalose
Physiology/genetics of yeast	The stationary phase is larger than the exponential phase Undefined cryoprotective factors

3.2.2. Metabolic engineering

The yeast strain is subjected to a variety of environmental factors during the baking process, including temperature, pressure, pH, osmotic water content, oxidation, and several chemical compounds. These intense conditions cause severe damage to organelles and cell membranes, which ultimately stops cell division or causes cell death. It becomes crucial to create or induce several cellular processes of stress adaption in order to live under this stress, such as the synthesis of stress proteins, beneficial modifications in membrane structure, and up- and down-regulation of appropriate gene expression. The ability to withstand stress can greatly enhance the growth of a yeast strain under fermentation, which will ultimately boost product yield [81]. It is crucial to enhance traditional baker's yeast utilizing cutting-edge methods like systems biology, metabolic engineering, and bioinformatics tools in addition to traditional methods like genetic and evolutionary engineering in order to get beyond these limitations. A schematic diagram of the various technologies used to improve baker's yeast is depicted in Figure 2. It has been noted that the majority of commercial strains of baker's yeast are polyploid in nature, lacking any mating behaviour, low sporulation, or extremely low spore viability. These problems have been largely addressed by developments in yeast genetics and genetic engineering protoplast fusion technology [81].

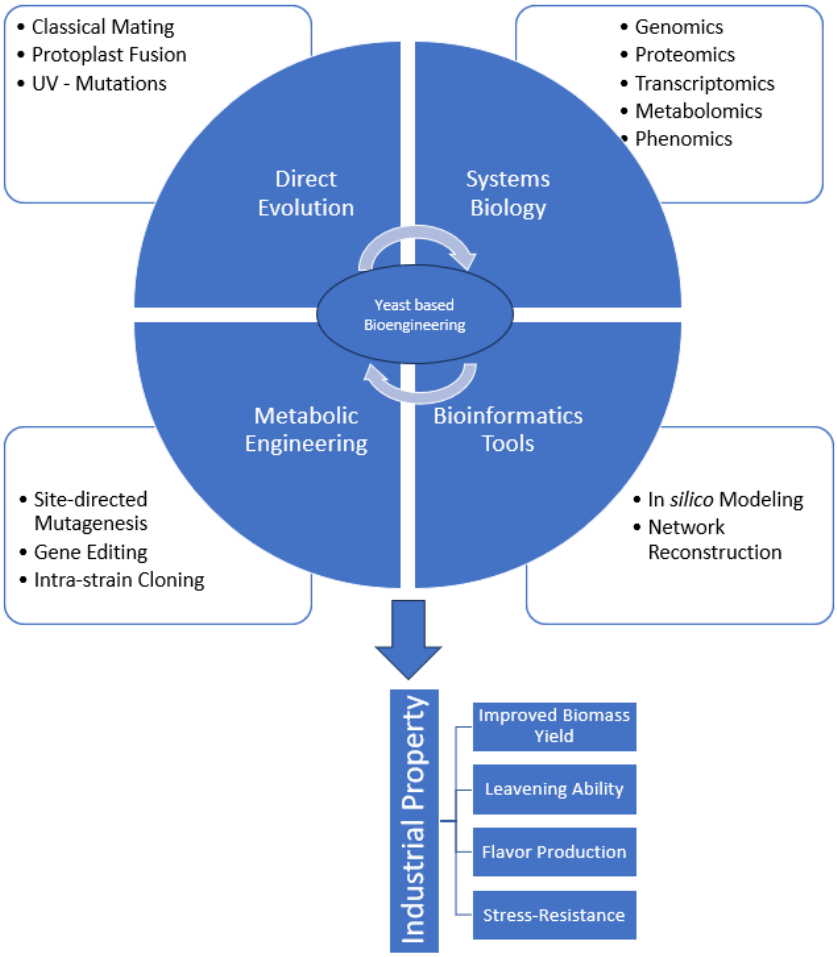

Fig 2. Using metabolic engineering to improve baker's yeast [81]

Metabolic engineering, the deliberate rerouting of metabolic fluxes has been extremely beneficial in enhancing yeast strains for all industrial uses. In contrast to conventional methods of enhancing genetic strains, such as selection, mutagenesis, hybridization, and mating, metabolic engineering offers two primary benefits: the deliberate alteration of strains without the accumulation of undesirable mutations and the incorporation of foreign organism genes to endow *S. cerevisiae* with unique characteristics [82]. Several microbial strains have been effectively improved through the use of metabolic engineering, a potent technique that produces metabolites that are not able to create naturally. Furthermore, this method allows bacteria to endure in harsh environments. Metabolic fluxes are directly altered by this method. Generally speaking, metabolic engineering uses recombinant DNA technology to modify the enzymatic, transport, and regulatory capabilities of the cell in order to manipulate biological pathways. It is literally true that metabolic engineering necessitates a thorough understanding of metabolic pathways since next-generation sequencing yields entire genome sequences of baker's yeast. Furthermore, an abundance of bioinformatics models and tools has been developed to analyze this genome-related data and provide comprehensive understanding of each metabolic pathway. Moreover, this data is utilized to build novel metabolic networks, which will be implemented in real life through a variety of molecular methods like genome and genetic engineering. Several reviews and research papers have been published for successful strain improvement using metabolic engineering [81].

4. Conclusions

Cryoprotectants are a technological advancement used in frozen dough to ensure the stability of the dough structure and to mitigate potential negative effects of frozen storage. Furthermore, these materials are being explored for the creation of novel baked goods with longer shelf lives while maintaining the industry-specific textural qualities. Knowing how these substances interact with different additives that are utilized to increase the dough matrix's palatability is one of the problems the company is now experiencing. It is critical to know if these things affect how cryoprotectants behave in frozen dough. Another challenge is the effect of individual or combination cryoprotectants on ice crystallization, which might lower the final product's quality.

Creating a product with acceptable physicochemical, microbiological, and sensory properties is a big challenge in the baking industry. The differential approach to cryoprotective chemicals for frozen dough in baked goods involves striking a careful balance between the need to preserve the finished product's quality and flavor and the necessity to protect the dough throughout the freezing process. Future research on cryoprotective materials for frozen dough in baked goods may focus on creating more sustainable, long-lasting, and environmentally responsible methods of extending the shelf life and caliber of these products. Mechanical damage happens during the technological processes used to generate frozen dough for bakery items. This damage reduces the specific volume and organoleptic qualities of the finished product, which has a substantial impact on its quality. Rapid starch retrogradation results in increased dough expansion, viscoelasticity, and hardness. Additives have been incorporated into the production process to enhance or maintain the quality of the dough throughout the baking stage and to increase the shelf life of the bread dough in an effort to manage the issues related to the storage of frozen dough.

Industry uses hydrocolloids, ice structure proteins, and polyols as additives; among these, polyols show the greatest promise as cryoprotectants because of their ability to integrate with the starch granules and gluten network in dough, as well as their protective properties. Even at higher prices, the addition of additives aids in ensuring longer product stability, and raw material reformulation can ensure industry technological advancement. Because of thawing technology, bakers, pastry chefs, and researchers are now considering cryoprotection as a feasible substitute to preserve the dough structure and guarantee the viability of yeast cells, producing a certain amount of final goods that are comparable to those made with processed yeast without freezing. Regarding baked goods, ice crystallization has a variety of uses, with the preservation of matrix integrity being its primary benefit. The industry has to keep researching cryoprotectant substances to learn how they behave and what impacts they have on various food matrices, as well as how they work in concert with other food additives.

5. References

[1]. KENIJZ, N., KOSHCHAEV, A., SOKOL, N., OMAROV, R., SHLYKOV, S., Products from Frozen Dough Pieces Using a Cryoprotectant as a Yeast Cell Stabilizer of Bakery Products from Frozen Dough Pieces Using a Cryoprotectant as a Yeast Cell Stabilizer. *Indo American Journal of Pharmaceutical Sciences Production of Bakery Production*, 6, *(*2019).

[2]. STRUYF, N., VAN DER MAELEN, E., HEMDANE, S., VERSPREET, J., VERSTREPEN, K.J., COURTIN, C.M., Bread Dough and Baker's Yeast: An Uplifting Synergy. *Comprehensive Reviews in Food Science and Food Safety,16*: 850–867, (2017).

[3]. LAHUE, C., MADDEN, A.A., DUNN, R.R., SMUKOWSKI HEIL, C., History and Domestication of *Saccharomyces cerevisiae* in Bread Baking. *Frontiers in Genetics*, 11, (2020).

[4]. YANG, Z., JIN, Y., XU, X., Mechanism of Frozen Dough Deterioration and the Behavior of Different Wheat Starch Types during Storage. *J Cereal SciJournal of Cereal Science*, 116, (2024).

[5]. MEZIANI, S., KACI, M., JACQUOT, M., JASNIEWSKI, J., RIBOTTA, P., MULLER, J.M., GHOUL, M., DESOBRY, S., Effect of Freezing Treatments and Yeast Amount on Sensory and Physical Properties of Sweet Bakery Products. *J Food EngJournal of Food Engineering*, 111: 336–342, (2012).

[6]. QIAN, X., GU, Y., SUN, B., WANG, X., Changes of Aggregation and Structural Properties of Heat-Denatured Gluten Proteins in Fast-Frozen Steamed Bread during Frozen Storage. *Food Chemistry, 365*: 130492, (2021).

[7]. MORIMOTO, N., SEGUCHI, M., Restoration of Breadmaking Properties to Frozen Dough by Addition of Sugar and Yeast and Subsequent Processing. *Cereal Chemistry*, 88: 409–413, (2011).

[8]. ZHANG, G., GUO, J., ZHAO, F., BAI, Z., ZHANG, L., Peptide Incorporation in Frozen Dough and Steamed Bread: Characteristics and Structure-Function Relationship. *LWT*, 205, (2024).

[9]. GUGERLI, R., BREGUET, V., VON STOCKAR, U., MARISON, I.W., Immobilization as a Tool to Control Fermentation in Yeast-Leavened Refrigerated Dough. *Food Hydrocolloids*, 18: 703–715, (2004).

[10]. DONGDONG, X., XING, L., YINGQI, S., SHUNCHENG, R., Effect of Different Producing Methods on Physicochemical and Fermentation Properties of Refrigerated Dough. *Journal of Food Composition and Analysis*, 119, (2023).

[11]. MAGALHÃES, F., CALTON, A., HEINIÖ, R.L., GIBSON, B., Frozen-

Dough Baking Potential of Psychrotolerant *Saccharomyces* Species and Derived Hybrids. *Food Microbiology*, 94, (2021).

[12]. PATKI, P.E., KUMAR SHARMA, G., SINGH BAWA, A., Role of Ingredients and Processing Variables on the Quality Retention in Frozen Bread Doughs: A Review. *Journal of Food Science and Technology*, 46(1): 12–20, (2009).

[13]. LIU, X., CHEN, L., CHEN, L., LIU, D., LIU, H., JIANG, D., FU, Y., WANG, X., The Effect of Terminal Freezing and Thawing on the Quality of Frozen Dough: From the View of Water, Starch, and Protein Properties. *Foods*, 12, (2023).

[14]. YANG, B., ZHANG, Y., YUAN, J., YANG, M., YANG, R., GU, Z., XIE, C., ZHOU, Q., JIANG, D., ZHOU, J., Impact of Different Frozen Dough Technology on the Quality and Gluten Structure of Steamed Buns. *Foods*, 11, (2022).

[15]. FENG, W., MA, S., WANG, X., Recent Advances in Quality Deterioration and Improvement of Starch in Frozen Dough. *Grain & Oil Science and Technology*, 3: 154–163, (2020).

[16]. KONDAKCI, T.; ZHANG, J.W.; ZHOU, W., Impact of Flour Protein Content and Freezing Conditions on the Quality of Frozen Dough and Corresponding Steamed Bread. *Food and bioprocess technology*, 8: 1877-1889, (2015).

[17]. LI, H., LV, Y., ZHANG, Y., WANG, X., LI, Z., QU, J., Improvement of the Freezing Resistance Characteristics of Yeast in Dough Starter. *Food Chemistry*, 458, (2024).

[18]. ARIAS, A.C., BOBADILLA, C.A.F., DOMÍNGUEZ, C.M.Z., Cryoprotectants for Frozen Dough: A Review. *Food Biophysics*,*19*: 18–28, (2024).

[19]. ZHANG, C., ZHANG, H., WANG, L., Effect of Carrot (*Daucus carota*) Antifreeze Proteins on the Fermentation Capacity of Frozen Dough. *Food Research International*, 40(6): 763-769, (2007).

[20]. YANG, S., JEONG, S., LEE, S., Elucidation of Rheological Properties and Baking Performance of Frozen Doughs under Different Thawing Conditions. *Journal of Food Engineering*, 284, (2020).

[21]. SHAN, S., CAMPANELLA, O.H., The Effects of Freeze-Thaw Cycles on the Rheological Properties of Yeasted and Non-Yeasted Frozen Bread Doughs. *Journal of Cereal Science*, 112, (2023).

[22]. RANDEZ-GIL, F., CORCOLES-SAEZ, I., PRIETO, J.A., Genetic and Phenotypic Characteristics of Baker's Yeast: Relevance to Baking. *Annual Review of Food Science and Technology*, 4: 191–214, (2013).

[23]. RIBOTTA, P.D., LEÓN, A.E., AÑÓN, M.C., Effects of Yeast Freezing in Frozen Dough. *Cereal Chemistry*,*80*: 454–458, (2003).

[24]. SHI, X., MIAO, Y., CHEN, J.Y., CHEN, J., LI, W., HE, X., WANG, J., The Relationship of Freeze Tolerance with Intracellular Compounds in Baker's Yeasts. *Applied Biochemistry and Biotechnology*,*172*: 3042–3053, (2014).

[25]. TORRANO, G.P., ATANAZIO-SILVA, G.A., BASSO, T.P., BASSO, T.O., TADINI, C.C., Development of Freezing-Resistant Hybrid Yeast from *Saccharomyces cerevisiae* for French Bread Dough. *LWT*,*199*, (2024).

[26]. HE, W., XUE, M., YU, H., ZHANG, X., LIN, Y., CHEN, Y., WANG, L., Co-Culture Fermentation Characteristics of Antifreeze Yeast and Mining of Related Freezing-Resistant Genes. *European Food Research and Technology*,*249*: 1161–1172, (2023).

[27]. MENG, L., YANG, X., LIN, X., JIANG, H.Y., HU, X.P., LIU, S.X., Effect of Overexpression of SNF1 on the Transcriptional and Metabolic Landscape of Baker's Yeast under Freezing Stress. *Microbial Cell Factories*, 20, (2021).

[28]. WANG, Z., TIAN, X., LIN, S., AN, Y., ZHAO, L., LI, M., Effects of Commercial Yeast on the Quality of Frozen Dough and

Steamed Bread in Different Regions of China. *Journal of Food Processing and Preservation*, 2023: 1–10, (2023).

[29]. MEZIANI, S., IOANNOU, I., JASNIEWSKI, J., BELHAJ, N., MULLER, J.M., GHOUL, M., DESOBRY, S., Effects of Freezing Treatments on the Fermentative Activity and Gluten Network Integrity of Sweet Dough. *LWT*, 46: 118–126, (2012).

[30]. MEZIANI, S., JASNIEWSKI, J., RIBOTTA, P., ARAB-TEHRANY, E., MULLER, J.M., GHOUL, M., DESOBRY, S., Influence of Yeast and Frozen Storage on Rheological, Structural and Microbial Quality of Frozen Sweet Dough. *Journal of Food Engineering*, 109: 538–544, (2012).

[31]. HALAGARDA, M., Effects of Trehalose and Dough Additives Incorporating Enzymes on Physical Characteristics and Sensory Properties of Frozen Savory Danish Dough. *LWT*, 86: 603–610, (2017).

[32]. SELOMULYO, V.O., ZHOU, W., Frozen bread dough: Effects of freezing storage and dough improvers. *Journal of Cereal Science*, 45.1: 1-17, (2007).

[33]. JIA, C., HUANG, W., WU, C., LV, X., RAYAS-DUARTE, P., ZHANG, L., Characterization and Yeast Cryoprotective Performance for Thermostable Ice-Structuring Proteins from Chinese Privet (*Ligustrum Vulgare*) Leaves. *Food Research International*, 49: 280–284, (2012).

[34]. ZHANG, B., OMEDI, J.O., ZHENG, J., HUANG, W., JIA, C., ZHOU, L., ZOU, Q., LI, N., GAO, T., Exopolysaccharides in Sourdough Fermented by *Weissella Confusa* QS813 Protected Protein Matrix and Quality of Frozen Gluten-Red Bean Dough during Freeze-Thaw Cycles. *Food Bioscience*, 43, (2021).

[35]. JIA, G., DU, Z., CHEN, Y., SUN, A., Quantitative Analysis Perspective: Ice Growth and Super-Chilling State of Frozen Dough under Quick Freezing. *LWT*, 179, (2023).

[36]. SUN, L., ZHU, Z., SUN, D.W., Regulating Ice Formation for Enhancing Frozen Food Quality: Materials, Mechanisms and Challenges. *Trends in Food Science & Technology*, 139, (2023).

[37]. ZHU, Z., ZHOU, Q., SUN, D.W., Measuring and Controlling Ice Crystallization in Frozen Foods: A Review of Recent Developments. *Trends in Food Science & Technology*, 90: 13–25, (2019).

[38]. KIM, I.S., CHOI, W., SON, J., LEE, J.H., LEE, H., LEE, J., SHIN, S.C., KIM, H.W., Screening and Genetic Network Analysis of Genes Involved in Freezing and Thawing Resistance in Damdhar—Expressing Saccharomyces Cerevisiae Using Gene Expression Profiling. *Genes*, 12: 1–27, (2021).

[39]. SHI, K., YU, H., LEE, T.C., A Novel Approach for Improving Yeast Viability and Baking Quality of Frozen Dough by Adding Biogenic Ice Nucleators from Erwinia Herbicola. *Journal of Cereal Science*, 57: 237–243, (2013).

[40]. OMEDI, J.O., HUANG, W., ZHANG, B., LI, Z., ZHENG, J., Advances in Present-Day Frozen Dough Technology and Its Improver and Novel Biotech Ingredients Development Trends—A Review. *Cereal Chemistry*, 96: 34–56, (2019).

[41]. PHIMOLSIRIPOL, Y., UBONRAT S., VANNA T., DONALD J.C., Effects of Freezing and Temperature Fluctuations During Frozen Storage on Frozen Dough and Bread Quality. *Journal of Food Engineering*, 84.1: 48-56, (2008).

[42]. LUO, W., SUN, D.W., ZHU, Z., WANG, Q.J., Improving Freeze Tolerance of Yeast and Dough Properties for Enhancing Frozen Dough Quality - A Review of Effective Methods. *Trends in Food Science & Technology,72*: 25–33, (2018).

[43]. DE LA CRUZ-GAVIA, A., PÉREZ-ALONSO, C., BARRERA-DÍAZ, C.E., ALVAREZ-RAMÍREZ, J., CARRILLO-NAVAS, H., GUADARRAMA-

LEZAMA, A.Y., Survival of *Saccharomyces cerevisiae* Microencapsulated with Complex Coacervate after Freezing Process. *Food Hydrocolloids*, 82: 45–52, (2018).

[44]. LI, Y., LI, C., BAN, X., CHENG, L., HONG, Y., GU, Z., LI, Z., Alleviative Effect of Short-Clustered Maltodextrin on the Quality Deterioration of Frozen Dough: Compared with Trehalose and Guar Gum. *Food Hydrocolloids*, 118, (2021).

[45]. LIU, M., SUN, L., QIN, G., ZHANG, J., WEI, Y., SHEN, Y., MA, H., DANG, W., LI, J., LIANG, Y., Elucidation on the Fermentation Capacity Improvement of Frozen Dough by Sanxan. *LWT*, 184, (2023).

[46]. SIM, S.Y., AZIAH, N., CHENG, L.H., Thermal and Dynamic Mechanical Properties of Frozen Wheat Flour Dough Added with Selected Food Gums; *International Food Research Journal*, 19(1): 333-340, (2012).

[47]. HAMED, A., RAGAEE, S., ABDEL-AAL, E.S.M., Effect of β-Glucan-Rich Barley Flour Fraction on Rheology and Quality of Frozen Yeasted Dough. *Journal of Food Science*, 79: E2470–E2479, (2014).

[48]. OZULKU, G., Monitoring the Dough Properties, Quality Characteristics and Volatile Compounds of Whole Wheat Bread Made by Different Sourdough Types during Frozen Storage. *Foods*, 13, (2024).

[49]. ADAMS, V., RAGAEE, S.M., ABDEL-AAL, E.S.M., Rheological Properties and Bread Quality of Frozen Yeast-Dough with Added Wheat Fiber. *Journal of the Science of Food and Agriculture*, 97: 191–198, (2017).

[50]. JIA, C., YANG, W., YANG, Z., OJOBI, O.J., Study of the Mechanism of Improvement Due to Waxy Wheat Flour Addition on the Quality of Frozen Dough Bread. *Journal of Cereal Science*, 75: 10–16, (2017).

[51]. WANG, P., YANG, R., GU, Z., XU, X., JIN, Z., Comparative Study on the Freeze Stability of Yeast and Chemical Leavened Steamed Bread Dough. *Food Chemistry*, 221: 482–488, (2017).

[52]. TAN, H., DONG, J., WANG, G., XU, H., ZHANG, C., XIAO, D., Enhanced Freeze Tolerance of Baker's Yeast by Overexpressed Trehalose-6-Phosphate Synthase Gene (TPS1) and Deleted Trehalase Genes in Frozen Dough. *Journal of Industrial Microbiology and Biotechnology*, 41: 1275–1285, (2014).

[53]. SHAFISOLTANI, M., SALEHIFAR, M., HASHEMI, M., Effects of Enzymatic Treatment Using Response Surface Methodology on the Quality of Bread Flour. *Food Chemistry*, 148: 176–183, (2014).

[54]. KANG, M.J., CHUNG, S.J., KIM, S.S., The Effects of Transglutaminase and Refrigerated Storage on the Physicochemical Properties of Whole Wheat Dough and Noodles. *Foods*, 10, (2021).

[55]. GUO, W.T., YANG, X.F., JI, Y.S., HU, B., LI, W.G., ZHONG, X.Y., JIANG, S.T., ZHENG, Z., Effects of Transglutaminase and Glucose Oxidase on the Properties of Frozen Dough: Water Distribution, Rheological Properties, and Microstructure. *Journal of Cereal Science*, 111, (2023).

[56]. LI, Y., ZHAO, F., LI, C., XIE, X., BAN, X., GU, Z., LI, Z., Short-Clustered Maltodextrin Provides Cryoprotection by Maintaining Cell Membrane Homeostasis of Yeast during Frozen Storage. *Food Chemistry*, 405, (2023).

[57]. WANG, F., XIONG, S., LI, X., YU, J., HUANG, Y., LIU, Y., Cryoprotective Effect of Silver Carp Muscle Hydrolysate on Baker's Yeast *Saccharomyces cerevisiae* and Its Underlying Mechanism. *Food Science & Nutrition*, 8: 190–198, (2020).

[58]. SHIMA, J., SUZUKI, Y., NAKAJIMA, R., WATANABE, H., MORIL, K., TAKAN0, H., Breeding of Freeze-

Tolerant Baker's Yeast by the Regulation of Trehalose Metabolism. *Japan Agricultural Research Quarterly*, 34(4): 257 – 260, (2000).

[59]. NAKAMURA, T., TAKAGI, H., SHIMA, J., Effects of Ice-Seeding Temperature and Intracellular Trehalose Contents on Survival of Frozen *Saccharomyces cerevisiae* Cells. *Cryobiology*, 58: 170–174, (2009).

[60]. KIM, Y.S., HUANG, W., DU, G., PAN, Z., CHUNG, O., Effects of Trehalose, Transglutaminase, and Gum on Rheological, Fermentation, and Baking Properties of Frozen Dough. *Food Research International*, 41: 903–908, (2008).

[61]. GIANNOU, V., TZIA, C., Cryoprotective Role of Exogenous Trehalose in Frozen Dough Products. *Food and Bioprocess Technology*, 1: 276–284, (2008).

[62]. STEFANELLO, R.F., MACHADO, A.A.R., PASQUALIN CAVALHEIRO, C., BARTHOLOMEI SANTOS, M.L., NABESHIMA, E.H., COPETTI, M.V., FRIES, L.L.M., Trehalose as a Cryoprotectant in Freeze-Dried Wheat Sourdough Production. *LWT*, 89: 510–517, (2018).

[63]. SEMKIV, M. V., TERNAVSKA, O.T., DMYTRUK, K. V., SIBIRNY, A.A., Effect of Trehalose and Glycerol on the Resistance of Recombinant *Saccharomyces cerevisiae* Strains to Desiccation, Freeze-Thaw and Osmotic Stresses. *Science and Innovation*, 14: 73–85, (2018).

[64]. SUN, X., ZHANG, J., FAN, Z.H., XIAO, P., LI, F., LIU, H.Q., ZHU, W.B., MAL62 Overexpression Enhances Uridine Diphosphoglucose-Dependent Trehalose Synthesis and Glycerol Metabolism for Cryoprotection of Baker's Yeast in Lean Dough. *Microbial Cell Factories*, 19, (2020).

[65]. STECCHINI, M.L., MALTINI, E., VENIR, E., DEL TORRE, M., PROSPERO, L., Properties of Wheat Dough at Sub-Zero Temperatures and Freeze Tolerance of a Baker's Yeast *(Saccharomyces cerevisiae)*. *Journal of Food Science*, 67(6): 2196-2201, (2002).

[66]. TANGHE, A., TEUNISSEN, A., VAN DIJCK, P., THEVELEIN, J.M., Identification of Genes Responsible for Improved Cryoresistance in Fermenting Yeast Cells. *International Journal of Food Microbiology*, 55(1–3): 259-262, (2000).

[67]. MIHALY COZMUTA, A., JASTRZĘBSKA, A., APJOK, R., PETRUS, M., MIHALY COZMUTA, L., PETER, A., NICULA, C., Immobilization of Baker's Yeast in the Alginate-Based Hydrogels to Impart Sensorial Characteristics to Frozen Dough Bread. *Food Bioscience*, 42, (2021).

[68]. CABRERA, E., WELCH, L.C., ROBINSON, M.R., STURGEON, C.M., CROW, M.M., SEGARRA, V.A., Cryopreservation and the Freeze–Thaw Stress Response in Yeast. *Genes*,11: 1–11, (2020).

[69]. MIHALY COZMUTA, L., NICULA, C., PETER, A., APJOK, R., JASTRZĘBSKA, A., MIHALY COZMUTA, A., Insights into the Fermentation Process of Fresh and Frozen Dough Bread Made with Alginate-Immobilized *S. cerevisiae* Yeast Cells. *Journal of Cereal Science*, 107, (2022).

[70]. CHEN, A., Enhancing Freeze–Thaw Tolerance in Baker's Yeast: Strategies and Perspectives. *Food Science and Biotechnology*, 33: 2953–2969, (2024).

[71]. SIMONIS, P., LINKEVICIUTE, A., STIRKE, A., Electroporation Assisted Improvement of Freezing Tolerance in Yeast Cells. *Foods*, 10(1): 170, (2021).

[72]. TANGHE, A.N., VAN DIJCK, P., THEVELEIN, J.M., Determinants of Freeze Tolerance in Microorganisms, Physiological Importance, and Biotechnological Applications. *Advanced in Applied Microbiology*, 53, (2003).

[73]. TANAHASHI, R., NISHIMURA, A., KAN, K., ISHIZAKI, N., FUJISHIMA,

S., ENDO, H., TAKAGI, H., Enhancing Freezing Stress Tolerance through Regulation of the Ubiquitin–Proteasome System in *Saccharomyces cerevisiae*. *Fermentation*, 10, (2024).

[74]. JUNG, K.M., PARK, J., JANG, J., JUNG, S.H., LEE, S.H., KIM, S.R., Characterization of Cold-Tolerant *Saccharomyces cerevisiae* Cheongdo Using Phenotype Microarray. *Microorganisms*, 9, (2021).

[75]. TANGHE, A., VAN DIJCK, P., COLAVIZZA, D., THEVELEIN, J.M., Aquaporin-Mediated Improvement of Freeze Tolerance of *Saccharomyces cerevisiae* Is Restricted to Rapid Freezing Conditions. *Applied and Environmental Microbiology*, 70: 3377–3382, (2004).

[76]. GERARDO-RODRÍGUEZ, J.E., RAMÍREZ-WONG, B., LEDESMA-OSUNA, A.I., MEDINA-RODRÍGUEZ, C.L., ORTEGA-RAMÍREZ, R., SILVAS-GARCÍA, M.I., Management of Freezing Rate and Trehalose Concentration to Improve Frozen Dough Properties and Bread Quality. *Food Science and Technology*, 37: 59–64, (2017).

[77]. HERDEIRO, R.S., PEREIRA, M.D., PANEK, A.D., ELEUTHERIO, E.C.A., Trehalose Protects *Saccharomyces cerevisiae* from Lipid Peroxidation during Oxidative Stress. *Biochimica et Biophysica Acta*, 1760: 340–346, (2006).

[78]. CHEN, B.C., LIN, H.Y., Deletion of NTH1 and HSP12 Increases the Freeze–Thaw Resistance of Baker's Yeast in Bread Dough. *Microbial Cell Factories*, 21, (2022).

[79]. DONG, J., CHEN, D., WANG, G., ZHANG, C., DU, L., LIU, S., ZHAO, Y., XIAO, D., Improving Freeze-Tolerance of Baker's Yeast through Seamless Gene Deletion of NTH1 and PUT1. *Journal of Industrial Microbiology and Biotechnology*, 43: 817–828, (2016).

[80]. HERNANDEZ-LOPEZ, M.J., PRIETO, J.A., RANDEZ-GIÍ´DEPARTMENT, F., Osmotolerance and Leavening Ability in Sweet and Frozen Sweet Dough. Comparative Analysis between *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* Baker's Yeast Strains. *Antonie van Leeuwenhoek*, 84: 125–134, (2003).

[81]. DANGI, A.K., DUBEY, K.K., SHUKLA, P., Strategies to Improve *Saccharomyces cerevisiae*: Technological Advancements and Evolutionary Engineering. *Indian Journal of Microbiology*, 57: 378–386, (2017).

[82]. NEVOIGT, E., Progress in Metabolic Engineering of *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews*, 72: 379–412, (2008).