

## Potentialities of using cryoprotectants in gluten-free frozen dough and microwave baking as an emerging technology

Potencialidades do uso de crioprotetores em massa congelada sem glúten e assamento em microondas como tecnologia emergente

Potencialidades del uso de crioprotectores en masa congelada sin gluten y horneado en microondas como tecnología emergente

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### **Abstract**

Disorders related to human health involving proteins that form the gluten network are increasingly in society, justifying the need to offer bakery products for this market niche. This study aimed to gather important information about the challenges involving technological production in the gluten-free bakery market. The approach encompasses the development of frozen dough for gluten-free baking, the use of cryoprotectants to maintain cell viability during the freeze-thawing step, the application of emulsifiers to improve the quality of the bread and the baking by an unconventional method using the microwaves. The introduction of frozen dough in the gluten-free bakery sector represents an interesting tool. However, these bread formulations require a higher amount of water compared to a traditional bread formulation (70 to 120 %), which makes the freezing and thawing steps extremely challenging, especially when it comes to the biopreservation of yeast cell viability. Cryopreservation with isolated and/or combined application of penetrating (glycerol) and non-penetrating (sucrose, fructo-oligosaccharide, trehalose, proteins and hydrocolloids) cryoprotectants promotes changes in the viscosity of the medium and is an excellent alternative for the maintenance of the microorganism responsible for the fermentation process. The appropriate choices of cryoprotectants, as well as the control of the freezing and thawing and baking processes, are important steps in the production line, to guarantee products with adequate technological and sensory qualities.

**Keywords:** Yeast; Ice crystals; Emulsifiers; Carbohydrates; Proteins; Celiac.

### **Resumo**

As desordens em relação à saúde humana envolvendo as proteínas formadoras da rede de glúten se encontram cada vez mais presente na sociedade, justificando a necessidade de oferta de produtos de panificação destinados a esse nicho de mercado. O presente estudo teve como objetivo o levantamento de informações importantes sobre os desafios envolvendo a produção tecnológica no mercado da panificação isenta de glúten. A abordagem engloba o desenvolvimento de massa congelada para panificação sem glúten, a utilização de crioprotetores para manter a viabilidade celular durante a etapa de congelamento e descongelamento, a aplicação de emulsificantes para melhorar a qualidade dos pães e o assamento por método não convencional utilizando o micro-ondas. A introdução de massas congeladas representa uma alternativa interessante no setor de panificação sem glúten. No entanto, as formulações destes pães necessitam de uma quantidade de água superior quando comparada ao pão tradicional (70 a 120 %), o que

torna as etapas de congelamento e descongelamento extremamente desafiadoras, principalmente quando se trata da biopreservação da viabilidade celular das leveduras. A criopreservação com aplicação isolada e/ou combinada de crioprotetores penetrantes (glicerol) e não penetrantes (sacarose, fruto-oligossacarídeo, trealose, proteínas e hidrocoloides) alteram a viscosidade do meio e são uma excelente alternativa para a manutenção do micro-organismo responsável pelo processo de fermentação. As escolhas adequadas dos crioprotetores bem como o controle do processo de congelamento e descongelamento e de assamento são etapas importantes da linha de produção, com o propósito de garantir produtos com qualidades tecnológicas e sensoriais adequadas.

**Palavras-chave:** Levedura; Cristais de gelo; Emulsificantes; Carboidratos; Proteínas; Celíacos.

### Resumen

Los trastornos relacionados con la salud humana que involucran proteínas que forman la red del gluten están cada vez más presentes en la sociedad, lo que justifica la necesidad de ofrecer productos de panadería para este nicho de mercado. Este estudio tuvo como objetivo recopilar información importante sobre los desafíos que implica la producción tecnológica en el mercado de la panadería sin gluten. El enfoque abarca el desarrollo de una masa congelada sin gluten, el uso de crioprotectores para mantener la viabilidad celular durante la etapa de congelación-descongelación, la aplicación de emulsificantes para mejorar la calidad del pan y el proceso de horneado por un método no convencional utilizando microondas. La introducción de la masa congelada en el sector de la panadería sin gluten representa una herramienta interesante. Sin embargo, las formulaciones de estos panes requieren una mayor cantidad de agua en comparación con una formulación de pan tradicional (70 a 120 %), lo que hace que los procesos de congelación y descongelación sean extremadamente desafiantes, especialmente cuando se trata de la biopreservación de la viabilidad de las células de la levadura. La criopreservación con aplicación aislada y/o combinada de crioprotectores penetrantes (glicerol) y no penetrantes (sacarosa, fructooligosacáridos, trehalosa, proteínas e hidrocoloides) promueve cambios en la viscosidad del medio y es una excelente alternativa para el mantenimiento del microorganismo responsable del proceso de fermentación. La adecuada elección de crioprotectores, así como el control de los procesos de congelación, descongelación y horneado, son pasos importantes en la línea de producción, con el fin de garantizar productos con adecuadas características tecnológicas y sensoriales.

**Palabras clave:** Levadura; Cristales de hielo; Emulsificantes; Carbohidratos; Proteínas; Celíaco.

## 1. Introduction

Gluten intolerance is composed of three major classes of different disorders (Roszkowska, Pawlicka, Mroczek, Balabuszek & Nieradko-Iwanicka, 2019). Each condition presents different symptoms to individuals who ingest gluten for a certain reason, for example, inadequate specifications on packaging labels, lack of knowledge about the types of flour that contains or does not contain gluten-forming proteins and the difficulty in the choice of products suitable for consumption (Cabanillas, 2020; Bender & Schönlechner, 2020).

Celiac disease is defined as an autoimmune enteropathy that affects individuals who have genetic predispositions, is characterized by the presence of specific serological antibodies such as anti-tissue transglutaminase (tTG) IgA, anti-endomysium IgA peptides (EMA) and anti-gliadin peptides-deamidated IgG (DPG) (Roszkowska et al., 2019). This autoimmune enteropathy is caused by the ingestion of gluten and is characterized by permanent intolerance to proteins found in wheat, barley, rye and triticale (Rocha, Gandolfi & Santos, 2016; Lopes, Pereira & Rezende, 2019) and through cross-contamination in oat. The effects caused by the presence of gluten in the body of celiac people are mainly attributed to intestinal malabsorption, due to villous atrophy as the main symptom and the worsening of intestinal permeability, which causes aggression and inflammation of the intestinal mucosa (Lombardi & Passalacqua, 2019; Bender & Schönlechner, 2020).

The effects on the body of celiac people can also be linked to other factors such as the genetic modifications of wheat, the use of the microbial transglutaminase enzyme and the high consumption of gluten-based products in the diet. The clinical manifestations of celiac disease may not only involve the gastrointestinal system, but also skin sensitivity, the nervous system, the reproductive system and the endocrine system (Lebwohl, Ludvugsson & Green, 2015; Lopes et al., 2019).

In addition to celiac disease, there is yet another group of individuals that deserves attention. To those who have sensitivity and allergy to gluten and those who have opted for adhering to gluten-free diets as a lifestyle or for accompanying the need to exclude gluten from the diet of someone from their social or family life (Cabanillas, 2020).

Among wheat hypersensitivity, non-celiac gluten sensitivity is the least well-defined. It is estimated that the prevalence

of this condition varies from 0.6 to 6 % of the population (Scherf, Brockow, Biedermann, Koehler & Wieser, 2016; Taraghikhah et al., 2020). Symptoms related to sensitivity occur several hours, or even a few days, after the consumption of products with gluten-forming proteins, presenting symptoms very similar to those of celiac disease, including gastrointestinal and extraintestinal complaints (Catassi, 2015; Mansueto et al., 2019). The treatment employed may vary according to the degree of sensitivity diagnosed, with most of the partial or total removal of gluten from the diet.

Wheat allergy is defined as an adverse immune response induced by eating wheat (not just gluten-forming proteins) that leads to digestive hypersensitivity (Roszkowska et al., 2019). Allergy is mediated by the presence and absence of antibodies called IgE. In some cases, this modulation is done in a mixed way (with the absence and/or presence of the antibody). This modular factor indicates how the allergy will manifest itself in carriers (Scherf, 2019; Catassi et al., 2017). Allergies to wheat mediated by the presence of IgE are the most commonly diagnosed and can be triggered by different methods of contact with wheat, the most common of which are: inhalation of flour or flour powder (respiratory allergy), skin contact (skin allergy), or direct oral intake of wheat products (food allergy to wheat and wheat dependent exercise-induced anaphylaxis), preventive treatment after the diagnosis of allergy mainly involves the total removal of wheat and its derivatives of the diet, avoiding the minimum possible exposure. In more acute cases, treatment with antiallergic agents such as antihistamines or corticosteroids is also common (Scherf et al., 2016; Scherf, 2019).

In this sense, there is a need to meet the demand of people who need for consumption of products free of proteins forming the gluten network. Vieira (2017) interviewed 374 people with celiac disease, 73.3 % of respondents reported having great difficulty in finding establishments that offer gluten-free options on the menu, 52.9 % pointed out a high lack of food alternatives in supermarkets and bakeries and 76.2 % pointed out that they had great difficulty in identifying the gluten-free foods available, as well as finding them in supermarkets. Therefore, increasing the offer of gluten-free products becomes increasingly essential, to meet the needs of consumers, since there is still a shortage in the quantity and quality of commercially available products. The main objective of this literature review was to demonstrate how the various factors can affect the quality of the final product and how challenging it is to implement new products in terms of process control.

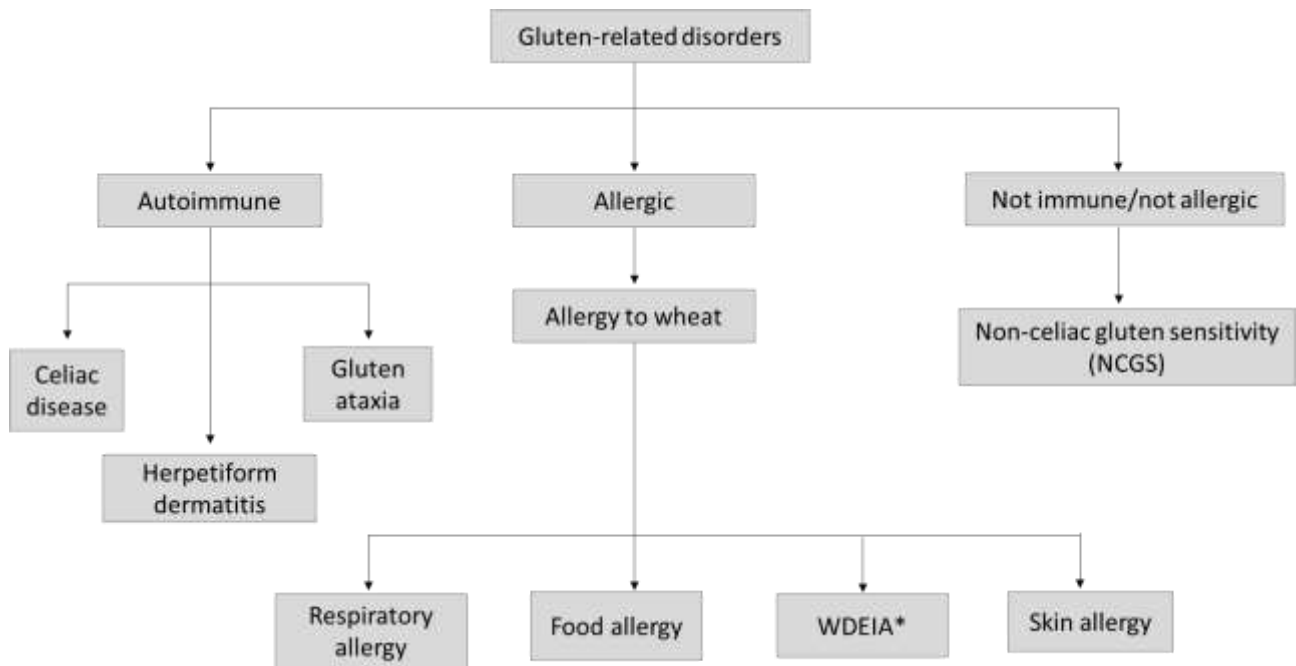
## **2. Methodology**

This study was conducted using a scientific methodology for integrative literature review and carried out in stages as proposed by Pereira, Shitsuka, Pereira and Shitsuka (2018). This article reports the main challenges encountered in the development of frozen doughs for gluten-free baking, addressing points that must be considered in the production process, to obtain products with satisfactory technological and sensory quality. The electronic databases used in the search for articles were: ScienceDirect (<https://www.sciencedirect.com/>), Scopus (<https://www.scopus.com/home.uri>), Scielo - Scientific Electronic Library Online (<https://scielo.org/>) and CAPES / MEC Journal Portal (<https://www.periodicos.capes.gov.br/>). The selected scientific articles were mainly from the period between 2011 and 2021, so that they contribute in an expressive and specific way to the research, excluding technical norms. Articles, books and book chapters with relevant information previous to this period were also considered.

## **3. Literature Review**

The gluten disorders are classified as (i) autoimmune, commonly represented by celiac disease (CD); (ii) allergic, represented by wheat allergy (WA); and (iii) non-autoimmune and non-allergic, represented by non-celiac gluten sensitivity (NCGS), as shown in Figure 1 (Roszkowska et al., 2019).

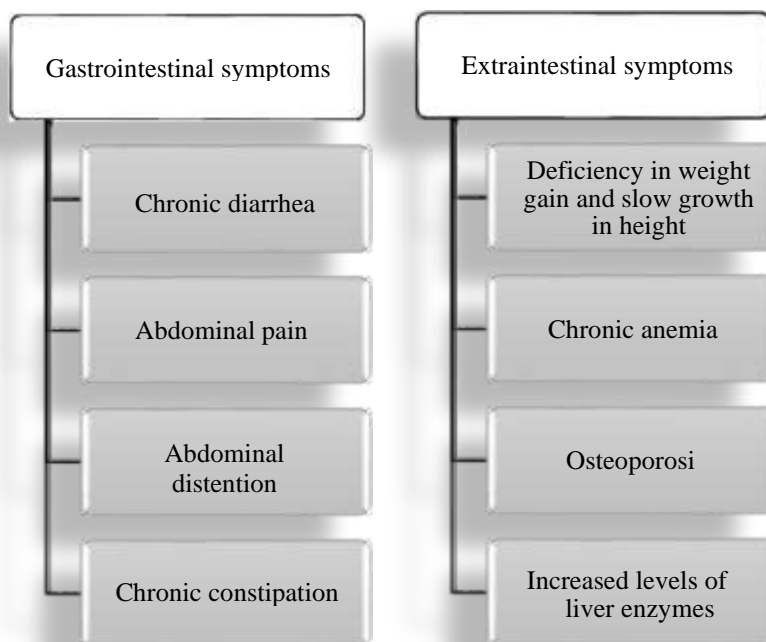
**Figure 1 - Celiac disease and gluten-related disorders.**



\*WDEIA - Wheat-dependent exercise-induced anaphylaxis. Source: Adapted from Cha e Kim (2020).

The celiac disease comes in four different forms: classic, non-classic, subclinical and asymptomatic. The traditional form is recognized in the first years of life, after introducing cereals containing gluten in the diet, presenting severe gastrointestinal symptoms such as intestinal malabsorption, recurrent diarrhea, and abdominal distention. The non-classic form is characterized by the presence of mild symptoms, such as digestive manifestations practically absent, being diagnosed late in children and adults. The subclinical condition, on the other hand, is characterized by extraintestinal symptoms as shown in Figure 2. In the asymptomatic form, there are positive serology and absence of symptoms (Green, Lebwohl & Greywoode, 2015; Balakireva & Zamyatnin Junior, 2016; Biesiekierski, 2017).

**Figure 2** - Gastrointestinal and extraintestinal symptoms of celiac disease.



Source: Adapted from Lopes et al. (2019).

### 3.1 Technological properties of gluten-free bread

The bakery market has grown significantly in recent years. Data published by the "Instituto Tecnológico de Panificação e Confeitaria (ITPC) - Technological Institute of Bakery and Confectionery - in 2020, shows the bakery sector among the six largest segments of the industry in Brazil, the growth projection presents favorable scenarios for the gluten-free market. According to the publication, the evolution index in the sector is expected to reach 3.5 to 4.0 % by 2022, with a turnover of approximately US \$ 35 billion per year for the field. The companies in the segment registered a 2.7 % growth in 2019, and sales reached R\$ 84.7 billion. Bread with substitutions of ingredients, with the use of whole ingredients or exclusion of some ingredients in the formulation, were the sectors of production that presented the greatest development compared to the traditional one (Khoury, Balfour-Ducharme & Joye, 2018).

Literature data that report quality parameters for gluten-free bread are still lacking. For comparison purposes, we chose to check the characteristics that consumers, in general, consider important for bread with gluten. In this regard, to identify the lack of studies that showed the preference of the national consumer for wholegrain or white bread (Ishida & Steel, 2015) evaluated the physical-chemical and sensory characteristics of twelve commercial loaves of bread, seven white and five wholegrains. It was observed that the samples showed a decrease in moisture content and water activity and an increase in the firmness of the crumb as the days of storage were prolonged. The sensory characterization was carried out in two distinct stages. In the first stage, the affective test was applied to gather information about the preference and frequency of consumption of the bread samples and the acceptance test to evaluate the sensory attributes about the appearance, aroma, flavor, texture and general impression. The results showed that the main factors that directly affected the purchase intention of these products were the flavor (19.6 %), the softness (16.8 %) and the expiration date of the product (14.3 %). The appearance attributes, the possible health benefits and the price appeared as secondary factors in the decision to purchase and consume the products. In the second stage, a survey of the purchase preference between white and wholemeal bread was performed, with 62.8 % of consumers opting for wholemeal bread, as it had a higher amount of dietary fiber compared to white bread.

Most bakery products are made up of ingredients that perform specific functions in the dough formation process. Although the constituents may vary in the manufacturing process, they all play a certain role. In this way, the ingredients are classified as basic and optional, the essentials (basic) in a bread formulation are flour, yeast, salt (sodium chloride) and potable water (Scheuer et al., 2016; Scheuer, Mattioni, Limberger-Bayer, Tatsch, Miranda & Francisco, 2017; Franco, 2015). However, other components can also be added to improve technological or sensory characteristics, aiming to improve the specific volume, the softness of the crumb and the texture and color of the crust, facilitating the incorporation of air into the dough or batter and increasing durability during storage, giving bread the desired characteristics (Franco, 2015). Thus, several technological parameters indicate the quality of the bread, the most used being the specific volume and texture.

The specific volume is one of the first parameters that indicate the quality of the bread produced and depends directly on the expansion of the volume of the dough during fermentation and in the baking process, through the phenomenon known as oven rise and oven spring (Packkia-Doss, Chevallier, Parea & Le-Bal, 2019). This parameter can be affected by the type of flour, amount of water absorbed by the dough or batter, interaction between hydrocolloids, presence of dietary fibers and proteins with aeration properties, emulsifiers, processing conditions and other ingredients, additives and technological aids present in the formulation or through appropriate technological processes (Scheuer et al., 2017; Salehi, 2019).

The production of bread without flours responsible for the development of the gluten network, presents a great challenge in obtaining a product with the appropriate specific volume, since, with the removal of gluten, the expansion of the dough in the proofing and baking steps are lower when compared to a traditional bread (gluten bread). This is because the gluten-free dough or batter has greater fluidity and less viscosity, due to the high amount of water needed in the formulation (70 to 120 %). Differences in the processing of gluten-free bread resulted in the development of a variety of ingredients and additives known as enhancers. Among the enhancers used, the importance of using hydrocolloids and emulsifiers is highlighted, which are used to assist in the construction and maintenance of the structure that will allow the proofing and baking process, resulting in gluten-free bread with technologically acceptable qualities, such as the improvement of the specific volume and softness of the crumb, the uniformity of color and the best nutritional quality (Brites, Schmiele & Steel, 2018).

Emulsifiers are an important class of complementary ingredients called additives, which are added to the formulations of gluten-free bread as a way of circumventing the technical problems caused by the absence of the development of gluten in the dough. The interaction with the dough occurs through the lipophilic portion present in the emulsifier, which interacts with the protein (gluten reinforcers) and starch (crumb softener) during fermentation and in the initial stage of baking. The complex formation between the emulsifier and protein occurs providing better retention of the CO<sub>2</sub> produced during the bread fermentation and in the baking process, this promotes improvements in the technological characteristics of the bread as the specific volume (Garzón, Hernando, Llorca & Rosell, 2018). In addition, emulsifiers also act as a dough softening agent, through its interaction with amylose, improving the stabilization of granules during the starch gelatinization process to delay water penetration, which contributes positively to texture-related parameters of bread as firmness and hardness in addition to improving the uniformity of the dough and crumbs (Nunes, Moore, Ryan & Arendt, 2009; Nabeshima, Hashimoto & El-Dash, 2003). Several types of emulsifiers are used in food-grade, with mono and diglycerides, lecithin and lactated esters being the main ones used by the food industry. Table 1 presents the main emulsifiers that are used as food additives classified as Generally Recognized as Safe.



**Table 1** - Most used emulsifiers in bread and gluten-free bread, classified as generally recognized as safe.

Emulsifier	Functionality
Distilled monoglycerides	Emulsifiers; aeration agent; crystallization stabilizer; dough reinforcers
Diacetyl tartaric acid esters of mono and diglycerides (DATEM)	Strengthen the dough; promote softness to the bread crumb; assist in water retention; reduce retrogradation levels; enhanced shelf life
Lecithin	Promote softness to the bread crumb; reduce retrogradation levels; enhanced shelf life
Polysorbates	Emulsifiers; solubilizers; moisture agent
Sorbitan esters	Aeration; lubricants

Source: Brites, Schmiele e Steel (2018); Orthoefer e Kim (2019).

The main characteristic of emulsifiers is the amphiphilic behavior, related to the hydrophobic and hydrophilic segments present in its chemical structure. Due to these characteristics, emulsifiers favor the migration of molecules between two physical phases, which reduces the surface tension in the interphase of immiscible phases, allowing them to mix, forming the emulsion or foam (Nunes et al., 2009; Castro, 2014). The lipophilic or hydrophobic region of the molecule will interact with the nonpolar lipid phase, while the hydrophilic region interacts with the polar aqueous components. This interaction between polar or non-polar regions is measured through the hydrophilic-lipophilic balance (HLB), which indicates the type of emulsion that will be formed (Stauffer, 1999; Nunes et al., 2009; Garzón et al., 2018). Higher HLB values favor the formation of hydrophilic oil/water emulsions and lower HLB values favor the formation of lipophilic water/oil emulsions (Zanin, Miguel, Chimelli & Oliveira, 2002; Oliveira, 2017; Cappelli, Oliva & Cini, 2020).

Emulsifiers can be classified according to their chemical structure and can be anionic, non-ionic, cationic and amphoteric. Anionic (i) emulsifiers, such as lactic acid esters, have an anionic charge in the hydrophilic region and when dissociating in water, they form negatively charged ions, being responsible for stabilizing oil/water emulsions (Nunes et al., 2009; Daltin, 2011; Oliveira, 2017). Non-ionic (ii) like distilled monoglycerides do not form ions in an aqueous solution and their solubility in water occurs due to the presence of functional groups that have an affinity with water. Cationic (iii) as well as anionic ones, present positive electric charge in the region hydrophilic, but they form positively charged ions when dissociated in the aqueous medium. Amphoteric (iv) such as lecithin and N-alkylamino acids present in their molecules both positive and negative charge, so according to the pH variation they may present an anionic character (pH between 9 and 10) or cationic (pH 4 to 9) (Nabeshima et al., 2003; Daltin, 2011; Oliveira, 2017).

The correct application of mixtures of emulsifiers, together with appropriate technological processes, result in important properties related to the quality of the bread, with the specific volume and crust and crumb texture being the most important parameters from the consumer's point of view. The texture can be assessed as a quality indicator through subjective assessments (sensory analysis) or objective assessments (instrumental texture) (Alencar, Steel, Alvim, Morais & Bolini, 2015; Scheuer et al., 2016). The methods of objective evaluation of the texture are measured through the texturometer, which evaluates desired attributes in the product, such as firmness, hardness and crispness. The most commonly applied tests are the instrumental texture profile and tension relaxation test. For the instrumental texture profile analysis (TPA) the measure consists of applying, on the same sample, one or two successive compressions that can cause reversible or irreversible changes in the bread crumb (AACCI, 2010). This evaluation is based on the reproduction of the chewing process. Among the main parameters considered in the analysis of instrumental texture are: (i) firmness and/or hardness, which is the force necessary to obtain a given deformation; (ii) elasticity, defined as the force at which a deformed material needs to return to the undeformed condition after removal of the deformation force; (iii) cohesiveness, the extent to which a material can be deformed before breaking;

(iv) chewiness, the energy required to crush a solid food to a state ready to be swallowed; and (v) resilience, which is given by the capacity or action of the bread crumb to return to its original state after the first deformation (Magalhães et al., 2019). In the stress relaxation test, a constant strain is applied. In this way, it is possible to observe the stress relaxation that the material presents, being able to be applied in different periods during the test, with the results being expressed through the deformation force, determined as a function of time (Silva, 2013).

### **3.2 Sensory properties of gluten-free bread**

Sensory analysis is a scientific discipline that has advanced significantly in recent decades, becoming an essential tool in predicting the success of a potential product on the market (Crofton, Botinestean, Fenelon & Gallagher, 2019; Andressa et al., 2020). When combined with the research and development, quality control and marketing sector, sensory analysis becomes indispensable in projecting the success and acceptability of consumers to the new product launched (Delaure, 2015; Kemp, Ng, Hollowood & Hort, 2018).

According to Meilgaard, Civille e Carr (2006), analytical methods are those that describe and quantify information about the characteristics evaluated. The assessment takes place in an objective manner, where the preferences and personal opinions of the tasters are not considered, being divided into discriminative (identify preferences) and descriptive (describe attributes) tests (Teixeira, 2009). Affective methods use untrained judges. The test principle is to point out the results of the testers' stimuli, perceptions and reactions when trying the tested product for the first time, determining its acceptability and/or preference (Stone, Bleibaum & Thoma, 2020). The descriptive sensory analysis encompasses the methods most used by the food industries to describe the characteristics of products, as well as to evaluate the characteristics of quality and acceptance (Moser, Lepage, Pineau, Fillion & Rytz, 2018).

The sensory characteristics of food are perceived by the organs responsible for the senses of sight, sound, smell, taste and touch. Sensory tests are used both to assess the quality of a product and to verify consumer acceptance (Moura, Canniatti-Brazaca & Silva, 2015). In bakery products with and without gluten, subjective texture, the color of the crust, aroma and flavor are sensory attributes commonly evaluated by consumers in the purchase decision process (Silva, 2020).

The texture approached subjectively through the application of sensory analysis was defined by Brandt, Skinner and Coleman (1963) as the sensory analysis of the texture of food, in terms of the mechanical, geometric, lipid and moisture characteristics, the degree with that each character is present and the order in which they appear from the first touch and subsequent bite until complete chewing, that is, they are the set of all rheological and structural properties (Teixeira, 2009). Mechanical characteristics are defined by primary and secondary parameters. The primary parameters are hardness and/or firmness, cohesiveness, viscosity, elasticity and adhesiveness; and the secondary parameters are fracturability, chewability and gumminess (Szczeniak, 2002). These parameters are very similar to those applied to the texturometer to simulate human behavior as closely as possible (Scheuer et al., 2016). The geometric characteristics are defined by the arrangement of the physical constituents of food, such as size, shape, presence of dietary fibers and soft or rigid particles, classifying them as fibrous, crystalline, or amorphous, or granular. The other characteristics are related to the content of lipids and moisture in the food, classifying them as oily or juicy (Szczeniak, 2002).

Color is the first contact of consumers with the product, showing great relevance to consumers. In addition to characterizing the appearance of the product, it also contributes to the purchase decision. Consumers tend to associate the color with odor, flavor, safety/quality and storage time, which directly correlates with the first sensory impressions of the product, once the product that has the darkest color, tend to receive the lowest grades, being associated with lower qualities. The color formation in bakery products during baking is a result of the Maillard reaction. In addition to the color, there is also the development of aroma and flavor characteristics of the bread (Scheuer et al., 2017). The taste is an attribute of great complexity,



where the mixture of different olfactory sensations dominates the characterization of this parameter. The taste is influenced by the tactile, thermal, painful and/or synesthetic effects, and this interrelation of characteristics is what differentiates one food from the other, since the aroma and the perception property of the aromatic substances of food after being placed in the food in the mouth (Teixeira, 2009; Morais, 2011; Moura et al., 2015).

To assess the perception of consumers concerning the quality aspect addressed above, there are studies (Table 2) that show how the application of the standards mentioned above, combined with the sensory analysis tools, can contribute to the evaluation of the quality and acceptability of products of bakeries with and without gluten.

**Table 2** - Research about technological and sensory quality aspects of bread with and without gluten.

Goal	Main findings	Reference
The authors evaluated the physical-chemical and rheological characteristics of the flour and the relationship of these properties to the quality of gluten-free bread. Six varieties of long-grain rice were used in the study for the production of flour	The properties of the flour showed a difference between the varieties of rice used to manufacture the flours. However, the length of the rice is not a determining factor for gluten-free breadmaking, long grain rice is also an alternative in gluten-free breadmaking. There was a significant positive correlation between the specific volume of the bread and the water absorption power parameters ( $r^2 = 0.71$ , $P < 0.01$ ) and the drop in viscosity of the paste ( $r^2 = 0.97$ , $P < 0.01$ ). Flours that showed the highest values in the water absorption index produced bread with adequate volume and firmness, the final gelatinization temperature ( $r^2 = 0.81$ , $P < 0.05$ ) showed a positive correlation with the specific volume. The flour samples did not show significant differences between them about the texture of the crumb. The authors observed that the rheological and physicochemical properties of the flour interfere on the final characteristics of the product	Cornejo & Rosell, 2015
Authors evaluated the effect of the hydrolyzed particle of guar gum (soluble fiber) and the level of water added in the formulation according to the parameters of firmness of the crumb, specific volume and sensory acceptance	The specific volume of the bread decreased with the increase in the substitution of flour for guar gum. The addition of guar gum in the bread formulation showed a significant positive effect ( $P < 0.01$ ) for crumb firmness and a significant negative effect ( $P < 0.01$ ) for specific volume and sensory acceptability. The level of water added showed a significant negative effect ( $P < 0.01$ ) for the firmness of the crumb and a significant positive effect ( $P < 0.01$ ) for specific volume, but it did not show any influence significant impact on sensory acceptability	Mudgil, Barak & Khatakar, 2016

Table 2. Continued...

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The sensorial characteristics of global acceptance were evaluated for color, flavor, texture, aroma, purchase intention and preference in three samples of gluten-free and casein-free bread made with carrots, bananas and pumpkin seeds using rice flour as a substitute for wheat flour	The bread evaluated showed satisfactory sensory characteristics in the color parameters of the crumb, which was the parameter that received the highest score, followed by the texture and flavor 7.27, 7.15 and 6.81, respectively. There was a significant difference ( $P < 0.05$ ) between the results of global acceptance, taste and texture for the three formulations. Pumpkin seed bread was the most preferred among the three samples (45 %) and also presented better responses regarding the intention to purchase the product (74 %)	Bergamo, Marinho, França & Lima, 2017
Authors evaluated the sensorial acceptance (appearance, color, flavor, texture and aroma) of gluten-free bread formulations developed with different levels of pumpkin seed flour and pumpkin peel flour, as well as determining the physical-chemical composition of the flours	The pumpkin seed flour showed higher levels of lipids, proteins, dietary fibers and calories, while the peel flour showed higher contents of moisture, ash and total carbohydrates. Considering the sensory attributes, the bread samples showed no significant difference between them ( $P < 0.05$ ), and the bread produced with the flour of the pumpkin peel obtained grades that ranged from four (I did not like or dislike) to seven (liked it regularly). The samples of the flour with the pumpkin seed received grades that ranged from six (I liked it slightly) to seven (I liked it regularly). For the purchase intention test, bread added from pumpkin seed flour performed better than absolute values when compared to bread with pumpkin skin flour, although there was no difference significant ( $P < 0.05$ ) between both	Anjos et al., 2017

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Source: Cornejo e Rosell (2015); Mudgil et al., (2016); Bergamo et al., (2017); Anjos et al., (2017).

### 3.3 Frozen dough

The quality of bakery products produced from frozen dough is widely influenced by the dough formulation and process parameters, such as freezing rate, time and storage temperature and thawing speed. According to Fellows (2017), freezing is the unitary operation whose objective is to conserve food, without significant changes in sensory quality or nutritional value, involving the reduction of the temperature of food below its freezing point. During the process, water phases change from liquid to solid in ice crystal forms (Jia, Yang, Yang & Ojobi, 2017; Zhang et al., 2020). Two important adverse consequences must be considered regarding freezing: (i) the aqueous components are concentrated in the liquid (not frozen) phase and (ii) there is an increase in the volume of about 9 % associated with the transformation of liquid water into ice, a phenomenon known as anomalous water dilation, as the freezing proceeds properties of the non-frozen phase such as pH and ionic strength will undergo modifications (Damodaran & Parkington, 2017).

The starting point of freezing a portion of food is described as the temperature where a tiny crystal of ice exists in

equilibrium with the water around it. During freezing, the sizes of the crystals formed can directly impact the cellular integrity of the food matrix. Parameters such as size, morphology and distributions of the ice crystals are directly related to the freezing rate conditions, affecting the nucleation and subsequent generation of ice crystals (Fellows, 2017). The freezing rate plays an important role in the final quality of the frozen products, determining both the number and the size of the ice crystals formed, two opposite effects can be observed (Akbarian, Koocheki, Mohebbi & Milani, 2016). At the rapid freezing rate ( $-20^{\circ}\text{C}$  in 30 minutes to 4 hours), ice crystals are formed, which do not affect the food matrices, providing adequate control of the crystallization process. At a slow freezing rate ( $-20^{\circ}\text{C}$  in 4 to 72 hours), the temperature gradually decreases until reaching the desired value, with the formation of larger ice crystals both inside the cell and in the intercellular spaces of the product, promoting distribution uneven in the food tissue, which can cause irreversible disruption of the cellular structure, causing sensory damage and loss of nutrients in the products (Damodaran & Parking, 2017; Kiani & Sun, 2011). In this way, the most regular crystals generated at high freezing rates lead to less damage to the food matrices, being, therefore, the adequate control of the crystallization process, essential for the success of the method (Liang et al., 2015; Zhu, Zhou & Sun, 2019).

The biggest challenge in the bakery is the production of a product with satisfactory technological quality. Physicochemical (softness loss of the crumb and water migration - crumb staling) and microbiological (molds) changes are the factors that most interfere with the shelf life of this product. Thus, production alternatives, such as par-baked bread and frozen doughs, are adopted to overcome this problem.

Par-baked bread is produced in a similar way to the conventional process, however, in the baking step, the product is partially baked. After this stage, the product is packed and stored and, only after the tawing process or directly introduced in the heated oven, is it submitted to cooking again to concluding the process (Bárceñas & Rosell, 2006). Due to the possibility of delaying this last re-baking step, this processing technology makes it possible to obtain fresh products at any time. Par-baked bread was an important driver in the bakery field, with the ease of receiving the bread ready to just be re-baked (Rosell & Gómez, 2007). The commercial establishments that use this product do not have to worry about specific areas of production and storage, the process reduces by up to 70 % the space needed for bread processing, requiring only a counter, oven and a small space to keep the frozen product (Almeida, Steel & Chang, 2014).

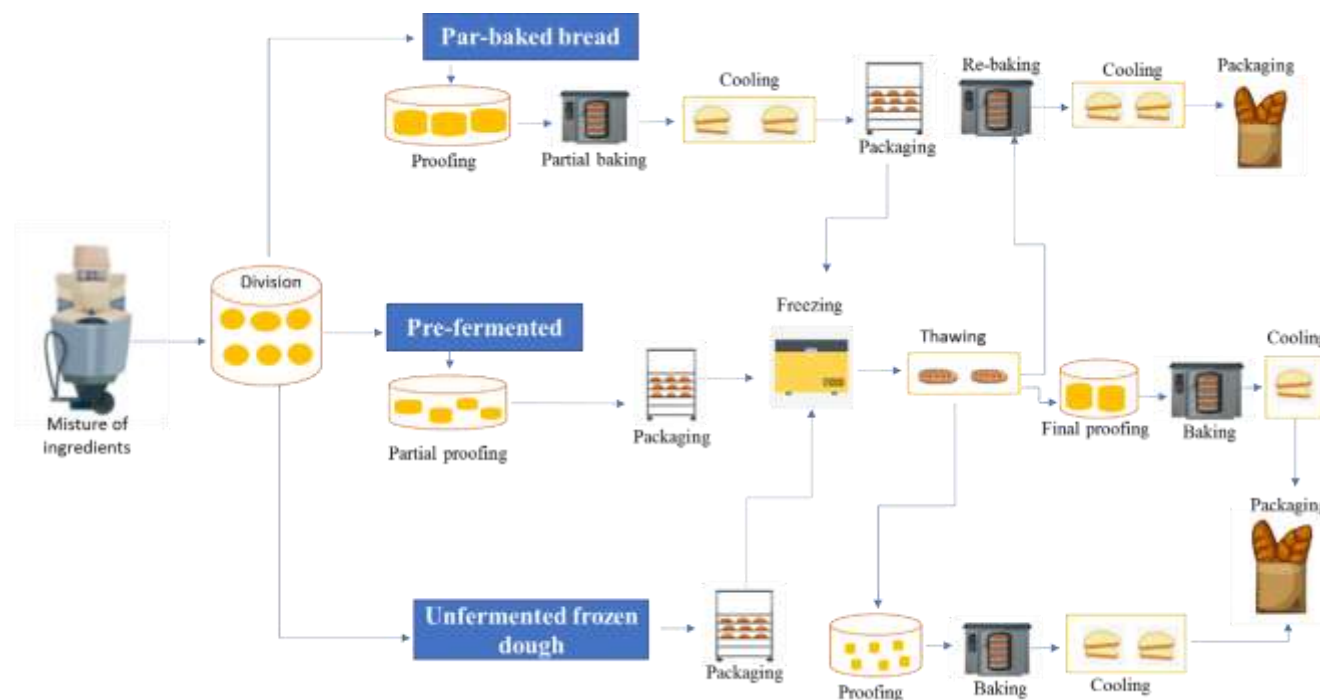
There are two types of frozen dough: pre-fermented and non-fermented. The pre-fermented dough has process stages like those of conventional baking, however, fermentation is stopped before the dough reaches full volume, followed by the freezing step. In this production process, high freezing rates are commonly employed, and the pre-fermented frozen dough is more susceptible to structural damage for the ice crystals compared to the unfermented dough (Lebail, 2006; Nur & Nilda, 2019). Besides, they require greater physical space for storage and demand greater resources for logistics and transportation, therefore, this technology is rare and little known in the bakery industry.

In unfermented dough (Figure 3), fermentation is carried out only after freezing and thawing the dough, in which the production process consists of the following steps: (I) the dough is prepared, (ii) molded, (iii) frozen, (iv) stored, (v) thawed, (vi) fermented and (vii) baked. In the production process of this type of dough, a slow freezing rate is usually be applied. However, there are limitations related to shelf life and increasing the time required for defrosting and proofing, to ensure that the dough reaches the desired volume before cooking (Nur & Nilda, 2019). One way of supplying the deleterious effects that both slow and fast freezing can cause on the structure of the dough and/or on microorganisms is the use of an intermediate freezing rate. In this type of freezing, the initial formation of ice crystals promotes saturation of the extracellular medium of microorganisms, allowing the migration of intracellular water to the external environment (greater concentration of solids), preventing the formation of ice crystals within yeast (Meziani et al., 2011; Meziani et al., 2012).

The technology of frozen dough in the gluten-free market has been gaining noticeable growth. The use of freezing appears like an excellent alternative for the production of bread, cheese bread, biscuits and cakes, offering excellent application

advantages, such as the extension of the shelf life and the prevention of starch retrogradation, characterized by the re-approximation of the molecules due to the reduction of temperature during cooling of the gelatinized starch, promoting the formation of intermolecular hydrogen bonds, since the syneresis and the release of water existing between the starch molecules polymer-polymer, the combination of these phenomena directly affects the shelf life of bakery products. Thus, freezing promotes an important development in the business model in the national and international bakery industry chain (Cafieiro, 2018; Yang, Jeong & Lee, 2020).

**Figure 3** - The general production process for par-baked bread and pre-fermented and unfermented frozen dough.



Source: Authors (2021).

The preservation of yeast vitality must be considered during the production of frozen gluten-free dough/batter, where the cryoprotectants could be a very viable alternative in the manufacturing process (Ortolan et al., 2015; Costa, Amaral, Santos, Gomes, & Schmiele, 2017). The control of this step is crucial for the success of the dough since in the formulation of gluten-free bread there is a greater addition of water concerning to traditional bread, about 70 to 120 % (on flour basis). The greater amount of water present results in more fluid batter compared to the traditional dough (with gluten). The additional water can damage the yeast cells in the crystal formation stage during the freezing and maintenance phase of the frozen dough. To solve this problem, it is usual to appeal to the use of cryoprotectants, which have the function of protecting yeast during the formation of ice crystals at low temperatures, around -18 to -40 °C (Damodaran & Parking, 2017). The choice of the appropriate cryoprotectant is important to ensure that the preservation of yeasts is efficient during the maintenance of the cold chain and defrosting of the dough.

### 3.4 Cryoprotectants

The term cryoprotectants emerged by the Society of Cryobiology in 1965, created to designate the component that offers cell survival after the freeze-thawing process (Kunsler, 2017). Yeast cells when rapidly cooled are susceptible to die due to the rapid formation of ice crystals in the environment. Without the proper cryoprotectant, the yeast cell membrane can rupture, which

would make biological functionality unfeasible after the thawing process (Bhattacharya, 2018).

The action of cryoprotectants on freezing is directly related to the formation of ice crystals and the modification of the freezing point and the glass transition. The behavior of each cryoprotectant is linked to the chemical composition, for example, disaccharides increase the glass transition temperature, which favors the stability of the product before freezing. Cryoprotectants are classified according to the mechanism of action, which may be ice nucleating agents, antifreeze proteins and compatible solutes (Nunes et al., 2015; Kenijz, Nesterenko & Zayats, 2019).

The ice-nucleating agents will interact with the available water, modifying the formation of the ice crystals. This formation mechanism prevents the uncontrolled growth of ice in the product to be preserved in plants, insects and bacteria. Despite being recognized as cryoprotectants, ice-nucleating agents have a more pronounced effect on single cells and not so much on biological tissues (Fuller, 2004).

Antifreeze proteins will decrease the freezing point without changing the melting point, superficially binding the small ice crystals that will prevent crystal growth and recrystallization (Fuller, 2004; Provesi, Valentim Neto, Arisi, & Amante, 2016). The study by Kenijz et al. (2019) points out the interaction of antifreeze proteins with water molecules, causing interference in the growth of ice crystals due to the formation of hydrogen bonds. These properties are general characteristics of antifreeze proteins, but the mechanism of action varies according to each type of protein (Li & Liang, 2015).

Compatible solutes are another class of cryoprotectants used in food. They are synthesized by plants (sunflower seeds and seaweed) and microorganisms (fungi) when subjected to stress. Such solutes are generally sugars (true sugars) and alcoholic sugars (derivates sugars), such as trehalose and glycerol, which interact with free water, altering the conformation of ice crystals and cell walls, preventing dehydration of cells (Kenijz et al., 2019; Kunsler, 2017; Motta, Paraguassú-Braga, Bouzas, & Porto, 2014).

Cryoprotectants can also be classified as intracellular (penetrating) and extracellular (non-penetrating). Intracellular (penetrating) cells act through colligative properties, leading to a reduction in the cryoscopy point. In this way, a greater amount of water remains in the liquid state at low temperatures, leading to a reduction in the intracellular concentration of solutes providing a less harmful environment to microorganisms during freezing. This behavior results in a decrease of water available for freezing, reducing the mobility of water molecules due to the higher viscosity of the solution, decreasing the freezing point (Sola, Oliveira, Feistel & Rezende., 2012; Damodaran & Parking, 2017).

Extracellular (non-penetrating) cryoprotectants are molecules capable of inducing an increase in the osmolarity of the external environment, promoting the outflow of water from the intracellular environment to the extracellular environment, thus preventing the formation of intracellular ice crystals during freezing (Hubálek, 2003; Fuller, 2004; Sola et al., 2012). These cryoprotectants are suitable for the preservation of microorganisms and tissues due to the ability to cover the cell surface, forming a viscous layer capable of stabilizing the cell wall and plasma membrane, reducing the possible damage caused by maintenance at low temperatures. The following stand out in this group: mono and oligosaccharides, mannitol, sorbitol, dextrans, methylcellulose, albumin and gums, among others (Hubálek, 2003; Zhang, Yao, Qi & Ying, 2020).

In addition to the classification as penetrating (intracellular) or non-penetrating (extracellular) cryoprotectants, these substances can also be differentiated by the viscosity provided to the medium. High viscosity cryoprotectants, such as propylene glycol and saccharides, act mainly in processes where rapid cooling rates are applied and are commonly used in the conservation of erythrocytes and bacteria (Costa et al., 2011). Low viscosity cryoprotectants, such as dimethyl sulfoxide (DMSO - C<sub>2</sub>H<sub>6</sub>OS) and methanol, provide inferior protection, precisely because they do not cause structural changes in biological material during freezing processes with rapid cooling rates (Morris, Goodrich, Acton & Fonseca, 2006; Costa et al., 2009; Sola et al., 2012). However, it is extremely important to mention here that not all the aforementioned cryoprotectants are GRAS (Generally Recognized as Safe).

The characteristics of carbohydrates (Table 3), such as solubility, molecular weight and glass transition temperature, directly interfere with preservation efficiency. The lower chain carbohydrates belonging to the mono, di and oligosaccharide groups, such as trehalose, sucrose, maltose and fructo-oligosaccharide exert a disruptive influence on the normal tetrahedral 3D structure of pure water (Damodaran & Parking, 2017). Thus, the protection mechanism acts on the freezing water portion of the solutions. When the water crystallizes, forming the ice, the concentration of solute present in the remaining liquid phase increases and the freezing point decreases, consequently an increase in the viscosity of the solution occurs (Stefanello et al., 2018). When the liquid phase solidifies into ice, cell mobilities become restricted and diffusion-dependent reactions decrease in speed. The restriction of mobility causes water molecules to be non-freezing, with no formation of intracellular crystals (Wong, 2018).

**Table 3** - Main physicochemical characteristics of carbohydrates.

Carbohydrates	Molecular weight (g/Mol)	Properties of anhydrate sugar		Properties of sugar in aqueous solution	Solubility in water (g/100g H <sub>2</sub> O)
		T <sub>m</sub> (K)	T <sub>g</sub> (K)	T <sub>m</sub> (K)	
Sucrose	342.3	465	325-343	241	204
Trehalose	342.3	476	350-352	243	69
Maltotriose	504.5	407	349	250	83
Maltose	342.3	402	316-368	243	83
Fructose	180.2	397	280-290	231	368

\*T<sub>m</sub> (K) = melting temperature; T<sub>g</sub> (K) = glass transition temperature. Source: Damodaran e Parking (2017).

The gums belong to the class of hydrocolloids, characterized by having properties of formation of viscous solutions and/or dispersions and gels under specific conditions. Thus, xanthan, guar and carrageenan gums have been studied as cryoprotectants in foods (Wong, 2018). Hydrocolloids act on the water absorption properties of the food matrix, decreasing the melting enthalpy, which reflects in the breakdown of some hydrogen bonds, limiting the freezing water content. This occurs due to the connection of free water, in this way the migration of water through the intracellular medium decreases which reduces the formation of ice crystals promoting the fall in the freezing point (Maity, Saxena & Raju, 2017). The decrease in the speed of formation of crystals in the intracellular environment reduces the cellular damage caused in the freezing process (Matuda, Chevallier, Pessôa, Le bail & Tadini, 2008; Damodaran & Parking, 2017).

Glycerol has a cryoprotective activity similar to that of trehalose (Huang, Wan, Huang, Rayas-Duarte & Liu, 2011). It penetrates the cell membrane through passive diffusion, remaining both in the membrane and in the cytoplasm (Aguiar et al., 2012). The protection mechanism occurs through the binding of glycerol molecules with water. It decreases the freezing point and limits the proportion of non-freezing water, reducing the amount of water available for the formation of ice crystals (Huang et al., 2011). However, its efficiency is limited by the amount added in the medium, where concentrations above 20% can exert toxic effects on the cells, causing physicochemical changes that can cause the plasma membrane to rupture and the removal of important proteins present in the membranes (Damodaran & Parking, 2017; Saeki, Farhat & Pontes, 2015). Table 4 shows some cryoprotectants allowed for use in food and the main characteristics.



**Table 4 - Main characteristics of cryoprotectants used in food.**

Cryoprotectant	Main characteristics
Trehalose	Glucose disaccharide, non-reducing, is formed by an $\alpha$ -D-glucopyranosyl unit and an $\alpha$ -D-glucopyranose unit and extracellular cryoprotectant. It has good water retention properties, stable, colorless, odorless and the substance most used as a cryoprotectant, the high cost of obtaining it hinders the commercial and research application
Sucrose	Oligosaccharide, non-reducing, composed of an $\alpha$ -D-glycopyranosyl unit and an $\beta$ -D-fructofuranosyl unit, extracellular cryoprotectant, presents high hydrophilicity and solubility, low molecular weight, appears as an interesting and accessible alternative in the application of cryopreservation processes
Fructo-oligosaccharides	Obtained through the enzymatic extraction and hydrolysis of chicory inulin ( <i>Chicorium intybus</i> ), producing short-chain fructo-oligosaccharides, soluble dietary fiber, has a prebiotic effect, greater solubility than sucrose, does not crystallize, does not precipitate, used in formulations of ice cream and dairy desserts
Xanthan gum	Polysaccharide produced by <i>Xanthomonas campestris</i> bacteria, soluble in hot and cold water, produces solutions of high viscosity, used as stabilizers in aqueous dispersions, suspensions and emulsions
Guar gum	Polysaccharide thickeners, obtained by grinding seed endosperm, have a higher viscosity compared to other gums, applied in dairy products and frozen desserts
Carboxymethylcellulose	Obtained from the Williamson* synthesis, an anionic polymer derived from purified wood pulp, widely used as food gums, when combined with xanthan gum and guar gum it can produce gels with high stability, used in the stabilization of protein dispersions
Glycerol	Glycerol (C <sub>3</sub> H <sub>8</sub> O <sub>3</sub> ) is a highly permeable polyhydric alcohol, intracellular cryoprotectant, has low molecular mass, has ease of permeabilizing the cell with the diffusion of 30 to 60 times slower than that of water

\*Williamson synthesis: it is the reaction of obtaining ethers by substituting halogens for organic halides for another negative group.  
 Source: Passos e Park (2003); Huang et al (2011); Ozkoc e Seyhum (2015); Maity et al (2017); Rosa e Cruz (2017); Bhattacharya (2018).

Previous work developed by our study group from the Integrated Lab of Cereals and Lipids (LICEL) located at the Federal University of Vales do Jequitinhonha and Mucuri (UFVJM) in Diamantina-MG, found promising results in the application of fructo-oligosaccharide, soy protein hydrolysate and yeast extract as cryoprotectants. The study developed by Teotônio et al. (2021) showed that the addition of soy protein hydrolysate to the bread dough achieved satisfactory preservation of important technological parameters, such as specific volume, hardness, moisture content and water activity when used in combination with yeast extract and/or fructo-oligosaccharide. The synergism between the cryoprotectants resulted in the non-penetrating effect, also improving the characteristics of the crumb, making it softer than the other samples. The softness of the kernels is a parameter of great importance for the choice of the product concerning consumers. Due to its great hydration capacity, soy protein hydrolysate promotes partial dehydration of the yeast, thus avoiding the freezing of water inside the micro-organism cell. This mechanism significantly decreases the damage caused to the cell wall and intracellular components, offering the cell adequate protection in the freezing and thawing steps (Gerardo-Rodríguez et al., 2017; Andrade, 2016; Tebben & Shen, 2018).

The work developed by Souza et al. (2017) demonstrated the application of fructo-oligosaccharide, soy protein hydrolysate and yeast extract as cryoprotectants applied as improvers in the paste properties of rice flour, widely used in the

gluten-free bakery as a substitute of wheat flour. Soy protein hydrolysate absorbs approximately 6 to 7 times its weight in water, limiting the availability of this solvent for the formation of the gel, resulting in an increase in the viscosity of the medium (Schmiele, Felisberto, Clerici & Chang, 2017), since the fructo-oligosaccharides and the yeast extract are water-soluble components and promotes a reduction in viscosity. In this way, the addition of soy protein hydrolysate to the flour contributes to the reduction of the amount of water available for absorption by the starch, increasing the peak viscosity. Rice flour with cryoprotectants showed higher values about the trend of relative retrogradation; however, the interaction between soy protein hydrolysate, fructo-oligosaccharide and yeast extract showed less syneresis, reducing the retrogradation of the system.

This factor is of great importance for the characteristics of bread after baking, as they are related to the rate of syneresis and retrogradation of starch, favoring the aging of bread. Lower rates indicate the extension of the product's shelf life and contribute to the maintenance of final quality. Also, the flour showed higher values of peak viscosity and lower values in the drop in relative viscosity, resulting in greater retention of CO<sub>2</sub> released by the yeast, helping to better expand the volume during the fermentation process of the dough. The study showed that the application of cryoprotectants favored the paste properties of rice flour gels.

### **3.5 Cryostabilizers**

There is a class of compounds that also act in the freezing process and they play the role of cryostabilizers. These are compounds that have large molecules and high molecular weight, such as polysaccharides and proteins. Unlike cryoprotectants, cryostabilizers do not alter osmolarity when frozen and do not affect the freezing point (Damodaran & Parking, 2017). Due to the high molecular weight, the stabilizing function of these substances acts mainly in increasing the glass transition temperature (T<sub>g</sub>) of a complex frozen system. The increase in T<sub>g</sub> results in a decrease in the T-T<sub>g</sub> difference, contributing to a reduction in transformation rates during storage. Another beneficial aspect of the addition of cryostabilizers is the narrowing of the temperature range in which the glassy (stable) frozen product with a hard texture, passes to a partially melted state, of smooth texture and maintains the desired quality for consumption (Pinedo, 2007; Zaritzky, 2010). Cryostabilizers are widely applied in the production of ice cream, due to their cryoprotective effect, inhibiting the growth of ice crystals during the freezing stage and preventing the recrystallization of ice and lactose during product storage (Maity et al., 2017).

### **3.6 Microwave baking**

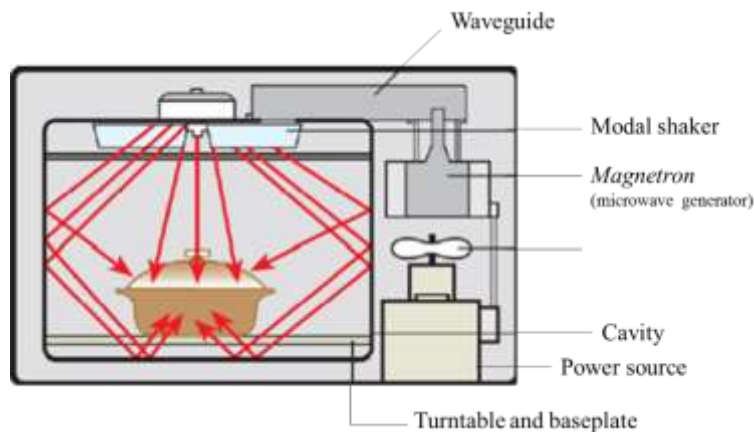
The cooking step of a bakery product is of great importance. During baking, physical, chemical and biochemical changes occur which promotes the development of the characteristic flavors of bakery products, in addition to being responsible for several changes related to the quality parameters of the bread such as volume expansion, water evaporation, starch gelatinization, protein denaturation, carbon dioxide production (fermentation process and oven rise phenomenon) and color formation through the development of the Maillard reaction and caramelization (Rosell, 2019; Pérez-Nieto et al., 2010; Purlis, 2010). When the dough is placed in a conventional oven previously heated for the beginning of the baking process, the hot air promotes the formation of a film on the surface of the bread. The expansion for gluten-free bread is directly associated with the starches present in the dough, and in the different gelatinization temperatures that promote the swelling of the starch granules leading to the expansion of the bread specific volume (Rosell, 2019; Garg, Malafrente & Windhab, 2019). In addition to the swelling of the starch, there occurs also an expansion of the gases which causes the growth of the dough. When the system stabilizes, golden color is formed on the crust resulting from the Maillard reaction and the caramelization of sugars. Together with the browning reactions occurs the production of pleasant aromas, flavors and tastes in the product (Sumnu, Sahim & Sevimli, 2005; Meda, Orsat & Raghavan, 2017).

Besides that, unconventional cooking methods have been gaining prominence in recent years. The adoption of the

microwave oven to heat food started between the 1970s and 1980s, applied as an unconventional way to bake products due to the convenience and low time required during this process (Barreto, 2017). The baking of the dough is considered a step of great importance in the production process, being responsible for several physical and chemical changes inside the product. According to Montebello, Araújo, Botelho & Borgo (2014), microwaves are generated by a magnet that transforms electrical energy into electromagnetic energy. The energy generated has a high non-ionizing frequency that transfers heat to the food immediately, favoring the modification of the thermal preparation operation. The heat transfer occurs inside the product convection and the penetration range of the microwave energy varies according to the composition of the food concerning the amount of water and temperature. The advantages of using the microwave as an unconventional way of baking are quick heating, short time, automatic process, quickly adaptable and easy to handle the equipment. Therefore, this technology is suitable for cooking various types of food (Fellows, 2017).

The adoption of the microwave oven to heat food started between the 1970s and 1980s. It was applied as an unconventional way to bake products, due to the convenience and low time necessary during this process (Calabró & Magazú, 2012; Barreto, 2017). According to Meda et al. (2017), microwaves are generated by a magnet (Figure 4) that transforms electrical energy into electromagnetic energy. The energy generated has a high non-ionizing frequency that transfers heat to the food immediately.

**Figure 4 -** General operation of a microwave oven.



Source: Adapted from Vollmer (2004); Bianchi (2018).

The propagation of heat can occur by two main transfer mechanisms, (i) dipole rotation and (ii) ionic conduction. The first is associated with the alignment of the molecules with the applied electric field, with the water present in the food being the main dipolar component responsible for the dielectric heating. The second heating mechanism occurs through the polarization of ions as a result of the back-and-forth motion of the ionic molecules, to align with the electric field, thus the heat is generated through frictional losses of these movements (James, James & Purnell, 2017; Bianchi, 2018; Yolacaner, Sumnu, & Sahin, 2019). The range of penetration of microwave energy varies according to the composition of the food concerning its quantity of water and temperature.

Some physical, thermal and electrical properties can affect the absorption of energy by the microwave oven and, consequently, interfere with the heating power of the food. Several authors (Ahmed & Ramaswamy, 2020; Bakr, 2020; Bianchi, 2018; James et al., 2017) listed some factors, which can directly influence the effectiveness of microwave heating:

- (i) dielectric properties: indicate the interaction of food with electromagnetic energy, demonstrating the behavior of food during heating in the microwave, being directly associated with the chemical composition of each food to be heated (Bakr, 2020);
- (ii) moisture content: the content of moisture directly affects the dielectric properties of the food, and consequently the depth of penetration of the waves. The uneven heating rate is observed in foods with high humidity, this factor is related to the low depth of penetration of the microwaves, as well as foods with low humidity present a more uniform heating rate, due to the deeper penetration of the microwaves. The initial moisture content of the product and the rate of moisture evaporation appears during microwave heating since the heating of the water depends directly on the proportion of the liquid water vs the solid ice phase and the free water content. available. At constant temperature, the dielectric behavior of free water remains constant in the lower frequency range (static region) and the water dipoles have enough time to reorient with little energy absorption, while a significant decrease in dielectric behavior can be observed. at higher frequencies (optical region) without field inversion by water dipoles. The dielectric constant decreases exponentially with the critical frequency between the static and optical regions. The phase change results in a significant change in the dielectric properties and, therefore, these properties for water and ice differ widely in their magnitude (Ahmed & Ramaswamy, 2020; Bianchi, 2018; Damodaran & Parking, 2017);
- (iii) temperature: Microwave heating is directly affected by the sample temperature. Dielectric properties may vary with temperature, depending on the material. Both the temperature and the moisture content can change during heating and therefore can have a combined effect on the dielectric constant. Freezing has an important effect on the heating capacity of a material due to the very different dielectric properties of ice and water. Water has a significantly higher value of dielectric constant and loss compared to ice, and these properties are also dependent on the frequency of microwaves. The initial temperature of the food to be heated must be controlled or known so that the microwave power can be adjusted to obtain uniform final temperatures (Ahmed & Ramaswamy, 2020);
- (iv) thermal properties: the heating characteristics of a given food will depend on the thermal characteristics, such as conductivity and thermal capacity. Materials with higher thermal conductivity dissipate heat more quickly than those with lower conductivity during microwave heating, foods that have a higher rate of thermal conductivity will take less time to reach a uniform temperature. The thermal conductivity of frozen foods is higher due to the high thermal conductivity of ice, while freeze-dried foods have lower thermal conductivity. The heat capacity of the food measures the response to the temperature of the food as a result of the entry or removal of heat. It is possible to increase the heat capacity by increasing the content of solids, adding components such as salt and proteins (Ahmed & Ramaswamy, 2020; Damodaran & Parking, 2017).

The application of the microwave oven as a way of baking has been showing constant growth due to the ease and convenience of its use. The main advantages associated with the use of the microwave as an unconventional way of baking are rapid heating, small equipment, automatic, adaptable and easy to handle, being suitable for cooking various types of food (Kachani, Castro & Fisberg, 2018). However, microwave ovens also have some disadvantages, such as careful control of cooking times. Furthermore, the food does not develop the characteristic color and crispness of the crust, when compared to conventional baking, since due to the accelerated cooking time it is not possible the development of the Maillard reactions and caramelization, which are mainly responsible for the characteristic color of the bread crust (Ramesh, 2020; Knoerzer, Regier & Schubert, 2017).

#### **4. Final Considerations**

The demand for products for individuals who have some type of disorder associated with gluten consumption has undergone significant growth in the current market. To achieve this niche of consumers, the frozen dough market has been

growing and gaining notoriety in this sector. However, for the development of this type of product, it is still necessary to improve the technologies applied in the production process. The control in the manufacturing process and the appropriate choices of ingredients are of great importance for the success in obtaining products that are technologically and sensorially well accepted by this target audience. Thus, cryopreservation emerges as a viable alternative in the development of frozen doughs for gluten and gluten-free breadmaking of for yeast leavened products which were submitted to freeze-thawing process, bringing a possibility of solution concerning the viability of yeast cells, one of the great challenges in obtaining gluten-free frozen dough.

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