

**Research Article**
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## Analysis of Frozen Bakery Items Containing Trehalose and its Impact on Quantitative Quality Measures

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### ABSTRACT

The frozen dough market has been increasing annually since it reduces cost while ensuring the availability of fresh products. Ice crystal formation inside the dough decreases yeast functionality while thawing releases fluid inside the dough, both leading to an unacceptable product. Trehalose can enhance the viability of yeast cells and reduce ice crystals. Four concentrations of trehalose (0, 10, 20, and 30%) were investigated on the freeze-thaw quality of frozen dough. Samples were prepared, frozen, and thawed before testing for color, moisture, water activity, and texture. Trehalose had no significant impact on color, moisture, or water activity of any sample despite trends in moisture and water activity being present. However, increased trehalose showed decrease in peak load in biscuits and pizza crusts. Therefore, more research is needed but data shows that trehalose could act as an anti-staling agent by reducing crust hardness in frozen biscuits and pizza crusts.

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### Introduction

Over the past decade, the frozen dough market has been rising at a steady pace. The market size of frozen dough is US \$18.59 billion in 2021 and will reach US\$ 27.44 billion by 2026. The economic benefits, such as labor cost and materials, have increased the food service industry interest toward using frozen dough products. The development of a frozen dough formulation that matches the quality of fresh baked products can reduce the cost of hiring specialized skilled workers in the food service industry but can ensure the availability of fresh bread. There are many benefits to both the manufacturer and customer since freezing and cold storage processes can deteriorate the quality of the frozen dough. Therefore, innovative ingredients and methods are being researched, such as the application of various ingredients to increase the freezing stability of yeast, and the introduction of hydrocolloids into dough systems to reduce drying out during frozen storage [1-7].

The quality of freezing dough is an important point of consideration for serving fresh bakery products. The formation of ice crystals inside the dough decreases the functionality of yeast cells while the thawing process melts the ice crystals releasing fluid inside the dough, both leading to an unacceptable product. Keeping yeast cells live with cryogenic food grade substances is an important strategy to improving the overall quality of frozen

doughs. Cryogenic carbohydrates have been widely employed as dough enhancers to decrease thawing loss, delay structural changes in gluten, and retain bioactivity of yeast in frozen dough. Cryogenic substances play an important functional role through the interactions of moisture in the dough or altering surface tension of ice crystals. These important determinants can affect the sensory attributes of the finished baked goods that in turn can affect the consumers' acceptability of the product [8-11].

One potential option for extending the shelflife and quality of frozen bakery products is the use of trehalose, a stable, colorless, odorless, non-reducing disaccharide that has been shown to exhibit 45% sensory sweet-taste characteristics compared to sucrose, as a partial sucrose replacement. Trehalose is considered generally recognized as safe (GRAS) for all food products except infant formulas by the US Food and Drug Administration (FDA) with no Acceptable Daily Intake (ADI) limit specified. Furthermore, almost all major international food regulatory authorities have evaluated trehalose for its safety and have approved its use in food applications with some recommending no more than 34.43g per day; above normal consumption habits [10,12-14].

Previous studies on the sensory application of trehalose have previously been demonstrated that trehalose constructively influences the products shelf life, texture, shape, and taste. Demonstrated that trehalose was the most suitable sucrose replacer because it resulted in similar rheological and sensory

properties as the sucrose-containing samples even after 12 weeks of storage. Furthermore, a study by found that sensory properties of sponge cakes by untrained panelists were strongly correlated to cohesiveness, adhesiveness, and springiness and trehalose did not have an impact on the raw product but led to finished product samples having high adhesiveness, which may limit its application as a 100% sucrose substitute in sponge cake. Additionally, demonstrated that a 25% sucrose replacement with trehalose showed they best initial and 28-day storage sensory scores for cakes when using a trained panel. Overall, there is no evidence of trehalose impacting the flavor of the finished product besides reduced sweetness and there is little published information on the impact of trehalose in frozen dough bakery products [13,15,16].

The application of trehalose in frozen dough products can enhance the viability of the yeast cells and prevent gas formation, to positively effect fermentation time and loaf volume. Many cryogenic food-grade substances have the ability to improve the quality of the freeze-thaw cycle and can prevent food waste. The moisture retention ability of trehalose is having also been recognized in the commercial baking industry. The suppression of foul odors is also a recognized trait of trehalose as it can interact with alpha-linolenic acid and suppress diacetyl formation and starch aging. The freshness of bread can be enhanced by preventing the loss of volatiles in the dough by trehalose. The commercially available dihydrate form of trehalose is characterized by low hygroscopicity, and the water content of the dihydrate crystals remain stable while exposed to a relative humidity of up to 92%. Dehydration of trehalose doesn't start until temperatures reach 970 C. This ability can improve the moisture retention capacity by limiting moisture immigration, ice recrystallization, and minimizing freeze-thaw degradation in frozen dough bakery products to improve the overall quality [8,17-19].

Additionally, Trehalose has been shown to interact with soy protein 11 S protein at the molten globular phase. Trehalose, a polyhydroxy carbohydrate, acts as an osmotic agent and its effect on protein stability have not been fully studied yet. However, it has been shown that the textural quality of dough with gluten proteins and trehalose reveals some evidence of an interaction. The freeze-thaw cycle of dough is affected by the hygroscopic nature of protein and the protein folding effect on dough quality [20].

With trehalose potentially affecting so many factors in frozen doughs, it is important to go back and look at the overall quality parameters when used in commercial formulas. Therefore, four different concentrations of trehalose were investigated on the freeze-thaw quality of frozen dough in four different bakery systems: yeast leavened baked (pizza dough), yeast leavened fried (yeast doughnuts), chemically leavened dough (biscuits), and chemically leaved batter (cake doughnuts).

## Materials and Methods

### Materials

Four bakery systems were made (biscuits, yeast doughnuts, cake doughnuts, and pizza crusts) using 0, 10, 20, and 30% trehalose as a replacement for the sugar in the formulas. The products were then baked or fried per the recipe and then frozen at -10°F for three weeks to match commercial foodservice storage conditions before thawing and testing for color, moisture, water activity, and texture (as peak load). All ingredients except trehalose (provided by Prinova US, LLC) were purchased through a local food service distributor.

### Biscuits

The control formulation was 4887.6 g buttermilk, 226.8 g salted butter, 340 g bread flour, 272 g cake flour, 38 g sugar, 28 g baking powder, and 15 g salt. Ingredients were mixed in a Kitchen-aid mixer until dough just came together (about 2-3 minutes). The mixture was then allowed to rest for 20 minutes. The dough was then rolled flat into a rectangle and 2 three-folds were performed. The dough was rolled out again into about a ½" to ¾" thickness and cut into 2" squares.

### Pizza Dough

The control formulation was 340 g all-purpose flour, 220 g water, 45 g high gluten flour, 10 g olive oil, 6 g salt, 6 g sugar, 6 g shortening, and 3 g dry yeast. All dry ingredients were combined in a Kitchen-aid mixer. Water was added mixing with a dough hook until dough just comes together. Oil and shortening were added and mixed until dough was smooth (5 minutes). Dough was left to ferment at room temperature for 2 hrs. with a punch down at the 1-hour mark. Dough was split into three equal parts and rolled into 8-9" disks before par-baking in a 450°F pizza deck oven.

### Cake doughnuts

The control formulation was 250 g all-purpose flour, 123.3 g apple juice concentrate, 115 g brown sugar, 115 g whole milk, 100 g sugar, 85 g butter, 50 g whole egg, 6 g baking soda, 5 g baking powder, 4.4 g cinnamon, and 1 g salt. Combine all dry ingredients in mixer bowl. In a separate container, mix sugars, with liquids, and eggs. Add in the cider and then mix in with the dry ingredients. Mix until just a smooth batter forms but no more (2-3 minutes). Pipe mixture into cake pans and bake at 375°F for 10-12 minutes.

### Yeast doughnuts

The control formulation was 175 g bread flour, 170 g whole milk, 115 g pastry flour, 56 g vegetable shortening, 38 g whole eggs, 20 g sugar, 9 g dry yeast, 5 g double acting baking powder, 5 g salt, and 4 g vanilla extract. In a Kitchen-aid mixer bowl, combine the liquid and dry ingredients. Using a dough hook, knead on low setting for 2 minutes. Add shortening and then continue to mix for 8 minutes in medium speed. Ferment for 2 hrs. at room temperature with a punch down to degas at the 1 hr. mark. Roll out to ¾" and allow to rest for 10 minutes. Cut doughnuts with ring cutter and proof until dough is springy, but not slack (about 10 minutes in proof box). Fry doughnuts at 350°F for 2 minutes per side.

## Methods

### Color

Samples were tested for L\*, a\*, and b\* using a tri-measure colorimeter (PCE Instruments, Model PCE-CSM3, Jupiter Florida). Reader was placed flat directly on to the sample to allow direct contact with sample and guard for a 10mm gap between sample and lens. Three (3), 2.5 cm diameter samples were taken from each product for analysis. Each reading collected was an average of 3 measurements by the colorimeter.

### Moisture

Samples were dried in a drying oven (Bio-Rad, Model BR-150, Hercules, California) for 24 hrs. at 75°C. Moisture was calculated as the difference in starting weight vs dried weight.

### Water Activity

Sample cups were half filled with each sample and then tested using standard procedure through a water activity meter (Meter Group, AquaLab Model 4TEV DUO, Pullman, Washington).

### Texture (as peak load)

Samples were tested for peak load in three unique spots using a single 15mm straight blade on a texture analyzer (Ametek Brookfield, Model CT3 10K, Middleboro, Massachusetts). The parameters for each test were a 1 second hold time, 5 g trigger, with a 10.0 mm deformation at 0.5 mm/second.

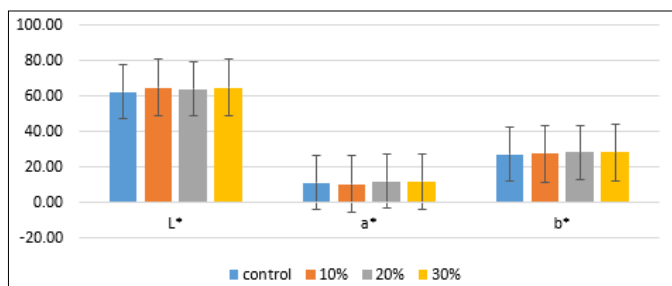
### Statistical Analysis

All analyses were run in nine replicate and compared using one-way ANOVA with the statistical difference identified using a t-test with the Bonferroni Correction at  $\alpha < 0.05$ .

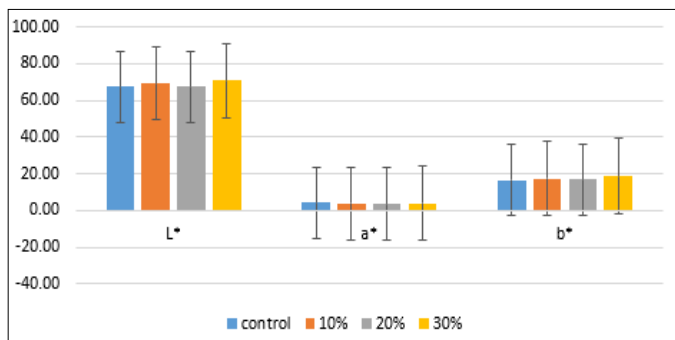
## Results and Discussion

### Color

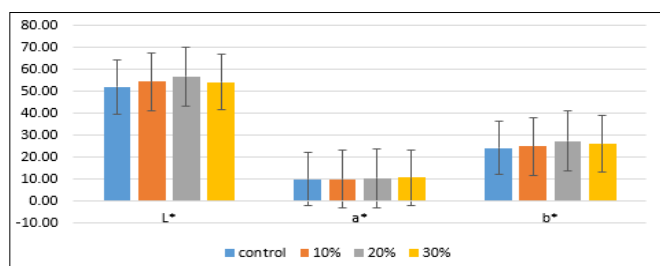
At the levels used in this study, trehalose had no significant impact on the finished color on any of the baked items as seen in Figure 1 below. However, showed that higher trehalose ratios applied to the cake led to an increase in the lightness ( $L^*$ ) and yellowness ( $b^*$ ) values of the cake crumb and crust while it reduced the redness ( $a^*$ ) factor [16].



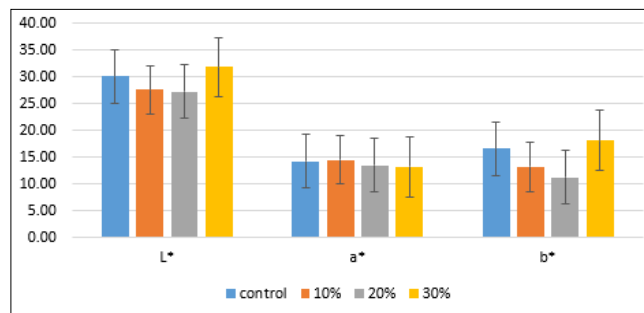
A: Biscuit Surface



B: Pizza Crust Surface



C: Yeast Doughnut Surface



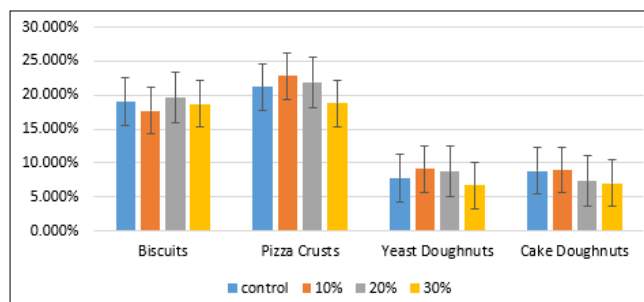
D: Cake Doughnut Surface

**Figure 1:** Surface Color Comparison with Varying Levels of Trehalose

It should be further noted that as the amount of trehalose was added to the samples, the researchers saw the overall crust color consistency improve which was also demonstrated by, when added to pan breads. It is well known that sugars cause a decrease in water activity by binding water through the “water replacement hypothesis”, or the ability of saccharides to bind onto molecular structure surfaces forming hydrogen bonds. In comparison to other disaccharides, trehalose has no direct internal bonds and are indirectly connected to the two molecules of water forming the native dihydrate structure. This feature causes unusual flexibility around the disaccharide bond allowing it to fit closer with the irregular surface of macromolecules in comparison to other disaccharides with a more rigid structure thus allowing for more stability at high temperatures. In this way, trehalose could increase sucrose’s ability to more consistently react through caramelization and Maillard color formation on the product’s surface [21-24].

### Moisture

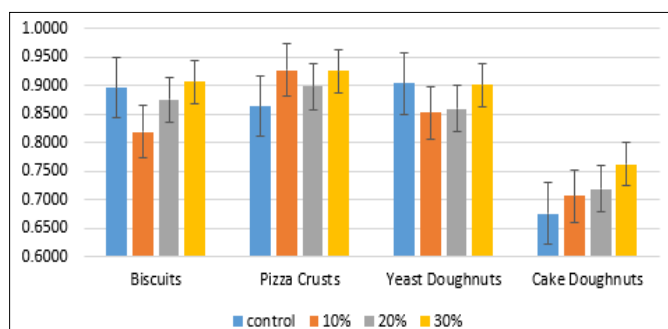
Moisture and water activity play a vital role in the sensory acceptability of baked items. Trehalose has shown the ability to retain water by hindering the interaction of water molecules, gluten, and starch, thus resulting in better water-holding capacity in baked goods. While there was no statistical difference between like samples, Figure 2 shows there was a general trend toward increased percent moisture with at lower levels of trehalose substitution in the pizza crusts, and yeast doughnuts but a decreasing trend in the percent moisture in all of the 30% samples. This is consistent with study which demonstrated that while sucrose has a high solubility in water, trehalose molecules have a higher water holding capacity due to their higher n(e-OH) interactions via hydrogen bonds [25,26].



**Figure 2:** Moisture of Baked Goods with Varying Levels of Trehalose

## Water Activity

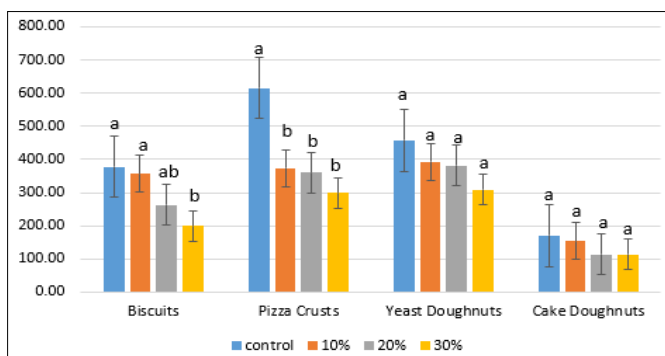
This would also explain why there was no statistical difference between like samples for water activity but still a general trend trehalose showed towards raising the water activity in all of the baked good samples especially at 30% replacement as seen in Figure 3.



**Figure 3:** Water Activity of Baked Goods with Varying Levels of Trehalose

## Texture (as Peak Load)

While most published literature focused on the impact of trehalose in terms of volume and or dough elasticity, very little information has been published on the textural qualities of the final baked foods. When trehalose levels were increased in the samples, there was a consistent decreasing trend for peak load texture in every product but there were only statistical differences in the biscuits and pizza crusts as seen in Figure 4 below [27,28].



**Figure 4:** Peak Load as an Indicator of Texture of Baked Goods with Varying Levels of Trehalose

The reduction in peak load in the biscuits and pizza crusts is a result of the trehalose holding onto moisture in these products. These results are in line with similar studies from and are consistent with the finding of Zhou et al. 2007 when they determined that trehalose reduced bread crumb hardness, the rate of firming, and performed better in sensory comparisons than a control [17,29-31].

## Conclusion

The US frozen pizza industry is only a \$6.0 billion dollar industry compared to the nearly \$47 billion US pizza restaurant segment Consumer Goods 2021. The significant difference in purchase volume is primarily due to overall quality difference per Consumer Goods 2021 report. Consumers want pizza crusts with flavor and a crisp yet chewy texture. Contrarily, frozen biscuits outsell refrigerated biscuits 5:1 due to shelflife and recent quality improvements. The data from this project, reveal that trehalose could act as a dough improver/anti-staling agent in frozen biscuits at 20% sucrose replacement and in frozen pizza crusts at 10% sucrose replacement. The practical implications of this work for

food operations, especially commercial bakery manufacturers, is the opportunity for increased quality, specifically reducing crust hardness, of their frozen bakery products compared to the competition. The slight increase in cost associated with using trehalose as a partial sugar replacer could be balanced with the benefits of improved sensory attributes and additional shelf life [32-35].

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## Declaration of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this article.

## Authors' Contributions

Darryl Holliday designed the work; Darryl Holliday & Alex Hammam prepared the samples; Darryl Holliday & Isabelle Giangrosso performed the laboratory analysis and collected the data; Darryl Holliday & Soma Mukherjee performed the data analysis and interpretation, and drafted the article, as well as the critical revision and final approval of the version to be published.

## Additional Information

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