




## Research Article

# Effect of Added Plant Hemicelluloses on the Stability of Frozen Bread Dough

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The use of hydrocolloids has greatly increased in recent years due to their pivotal role as functional ingredients. They can increase food consistency, control the microstructure that affects water absorption, alter texture and flavor, and improve the shelf life of baked products. In this work, we analyzed the effect of plant hemicelluloses such as *Caesalpinia pulcherrima* galactomannans and *Tamarindus indica* xyloglucans on the stability of frozen French bread dough. The amount of these additives used was optimized from an obtained central composite design- (CCD-) response surface methodology (RSM) of the alveograph parameters. Batches were characterized for moisture, water activity ( $a_w$ ), and texture using SEM, DSC, and TGA analyses when frozen for up to 60 days. Batches containing hemicelluloses showed better stability for  $a_w$  over time. There was no difference between the texture parameters of the samples studied for 60 days. Both added hemicelluloses presented fewer fractures at 30 days and less wear at 60 days, in addition to better performance in the TGA analysis after less than 30 days of frozen storage. Batches containing only xyloglucans or galactomannans had a higher solidification peak temperature after 60 days. Both plant hemicelluloses reduced the damage caused by cold storage and improved stability for water activity. Also, dough preparations containing these additives showed better moisture retention, as well as less wear and tear and fewer fractures over their shelf life. Our evaluation suggests *C. pulcherrima* and *T. indica* are nonconventional sources of hydrocolloids that could be utilized in the bakery industry.

## 1. Introduction

The food industry has experienced a significant increase in the use of hydrocolloids in recent years, mostly due to the influence of these biopolymers on the textural

and organoleptic properties of food products. Hydrocolloids can perform functions, including thickening and gelling aqueous solutions, stabilizing foams, emulsions, and dispersions, inhibiting ice and sugar crystal formation, and controlling the release of flavors, even at low

(<1%) concentrations [1, 2]. Among such hydrocolloids, plant hemicelluloses, such as galactomannans and xyloglucans, are polysaccharides with industrial applications due to their rheological properties in aqueous systems [3].

Hydrocolloids, such as plant hemicelluloses, present long chains of  $\beta$ -(1 $\rightarrow$ 4)-linked backbones with equatorial configurations and high molecular weights. Their sugar unit compositions differ depending on the plant species and extraction process [4, 5]. Hemicelluloses are closely associated with cellulose and lignin and contribute to the rigidity of plant cell walls in lignified tissues. They are classified as xyloglucans, xylans, mannans, glucomannans, and  $\beta$ -(1 $\rightarrow$ 3, 1 $\rightarrow$ 4)-glucans. The physicochemical characteristics and functional properties of such hydrocolloids depend on their molecular mass and degree of polymerization, branching, and macroscopic structure [3, 6].

Within the breadmaking process, hemicelluloses could be a potential alternative for hydrocolloids due to their many beneficial properties, which include antistaling, texture modification, and water retention, which confers stability during freeze-thaw cycles. Indeed, the staling effect in bread manufacturing is one of the largest current problems for the industry, as it shortens the shelf life of baked goods. Hydrocolloids can increase the gelling effect of starch and are used as dietary fiber, as they have shown the ability to mimic fat in different products [7]. *Tamarindus indica* and *Caesalpinia pulcherrima* are rich sources of xyloglucan and galactomannan hydrocolloids, respectively. While *T. indica* seeds are the major byproduct of the tamarind industry, the *C. pulcherrima* species represents a new potential source of hydrocolloids to use in the food industry. The former has xyloglucan polysaccharides in its thickened cotyledonary cell walls, while the latter contains galactomannan in its endospermic cell walls. Both species produce hemicelluloses with a yield of 25% of the seed mass [8].

In contrast, freezing technology has been applied to retard the deterioration of bakery products, preventing undesirable changes and extending their shelf life. A centralized manufacturing and distribution process that enables standardizing product quality has an economic advantage [7]. However, frozen products are usually inferior when compared to fresh equivalents. Several problems have arisen from bread produced from frozen dough because the process reduces the viability of yeasts and affects gluten crosslinking, causing a loss of dough strength. These drawbacks affect the CO<sub>2</sub> retention and fermentation time, yeast activity, and loaf volume and cause deterioration of the texture of the final product [9, 10]. In addition, hemicelluloses have been satisfactorily used as additives to the breadmaking process due to their ability to interact with gluten proteins, which improves the loaf weight, volume, crumb grain characteristics, crumb moisture, firmness, and rheological properties of the dough [11, 12].

Therefore, our study is aimed at assessing the effect of adding *C. pulcherrima* galactomannans and *T. indica* xyloglucans on the stability of frozen French bread dough. The amount of plant hemicelluloses chosen to be added to the formulations was determined by a response surface design methodology, considering the alveograph parameter (W,

the gluten strength). The moisture, water activity ( $a_w$ ), texture, scanning electron microscopy (SEM) images, differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA) of the dough preparations were compared, which suggested that these species are good nonconventional sources of hydrocolloids for use in the food industry.

## 2. Material and Methods

**2.1. Plant Materials.** *Caesalpinia pulcherrima* seeds were collected within Metropolitan Fortaleza, Ceará, while *Tamarindus indica* seeds were obtained from the Federal University of Ceará campus (UFC). Representative specimens were deposited in the Herbarium Prisco Bezerra, Federal University of Ceará, with numbers 56.367 and 56.965 for *C. pulcherrima* and *T. indica*, respectively. A commercial breadmaking mix was acquired from Grande Moinho Cearense and Frozen Bread Dough Industry (IMAC), both located in Fortaleza, Ceará, Brazil.

**2.2. Hemicellulose Extraction and Wheat Flour Characteristics.** Galactomannans and xyloglucans were extracted from endosperms of *C. pulcherrima* and cotyledons of *T. indica* seeds, respectively, through aqueous extraction followed by ethanol precipitation according to previously reported by [13]. The final precipitate was milled and passed through a 0.074 mm mesh. The moisture and ash content of galactomannan and xyloglucan were obtained according to [14]. The total protein content of the galactomannan was estimated by the Kjeldahl method for total nitrogen content using the conversion factor of 5.7.

The wheat flour was submitted to physicochemical characterization according to the Association of Official Analysis Chemists International standard [15]. The moisture and ash contents were determined according to the 44-15 and 08-12 gravimetric methods, respectively. The color was determined according to the 14-22 method using the lightness coordinate (L\*). Washing wheat flour with a 2% salt solution was used to determine the wet gluten content, as described in the 38-12 method. The wet gluten index was expressed as a percentage on a moisture basis. The falling number was used as an index of  $\alpha$ -amylase enzyme activity in the wheat flour samples and expressed in time as seconds (56-81B method).

**2.3. Preparation of Frozen French Bread Dough with Hemicelluloses.** In order to define the formulations, the combined effect of galactomannan and xyloglucan (two independent variables) was applied to assess the influence of added hemicellulose on wheat flour quality. Eleven treatments were defined, including four factorial points ( $\pm 1, \pm 1$ ), three central points (0, 0), and four axial points ( $(\pm\alpha, 0)$  and  $(0, \pm\alpha)$ ), according to variable levels in Table 1 [16]. A second-order central composite rotatable design (CCRD) and the response surface methodology (RSM) were used to analyze the dependent response variables for the alveograph parameter, W (gluten strength).

The dough formulations were optimized based on the results of the alveograph tests. The amount of hemicellulose added to the samples was determined using the valid

TABLE 1: Variable levels of percentage of hemicelluloses used in the experimental design.

Variable	$\alpha = -1.41$	-1	Level 0	+1	$\alpha = +1.41$
Galactomannan (Gal)	0	0.075	0.25	0.425	0.50
Xyloglucans (Xilo)	0	0.075	0.25	0.425	0.50

mathematical model ( $R^2 = 0.8663$  and  $F_{cal} = 15.87 > F_{tab} = 4.35$ ), considering the gluten strength to be 300 ( $W = 300$ ), which is classified by Brazilian legislation as a wheat improvement [17]. A mathematical model (Equation (1)) was used to predict the responses within the range studied.

$$W = 308.34 + 11.98(\text{Gal}) - 18.90(\text{Gal})^2 + 5.90(\text{Xilo}) - 3.71(\text{Xilo})^2 - 26.00(\text{Gal} \times \text{Xilo}). \quad (1)$$

The dough base formulation was then obtained by adding 55.00% water, 1.80% salt, and 0.20% Fleischmann's bread booster to 100.00 g of wheat flour. Fleischmann's Bread Booster was purchased in the local market and was added in order to enhance the dough preparation. The control formulation containing no added hemicelluloses was called HC. Three other batches were prepared by mixing 0.28% galactomannan (HG), 0.48% xyloglucan (HX), and 0.18% galactomannan and 0.24% xyloglucan (HXG) to this dough base. None of the preparations had yeast added to suppress the effect of the activity of baker's yeast on the thermal analyses.

A conventional dough process was used to prepare the bread samples. The ingredients were slowly mixed for 5 min to promote aggregation and then quickly mixed for 5 min until the dough was completely homogenized. Water was added at 16°C, and the temperature was later raised to 30°C ± 0.5°C. The dough was formed into a cylinder, molded, and frozen in a blast freezer at -24°C for 90 min following its gluten development. Next, the samples were packed in plastic bags and stored in a cold room at -18°C for up to 60 days.

**2.4. Influence of the Added Hemicellulose on the Characteristics of Stored Bread (Shelf Life).** The physical and physicochemical parameters of the samples were evaluated at time zero (fresh dough) and 5, 30, and 60 days of frozen storage. The moisture content loss was monitored according to the accepted 44-14 method reported by the Association of Official Analysis Chemists International [15]. Water activity was determined at a constant temperature (25°C ± 0.30°C) using Decagon CX-E equipment. Samples were thawed 20 min prior to analysis.

**2.5. Texture Profile Analysis (TPA).** TPA was carried out using a TA-XT2 texturometer with a cylindrical aluminum probe (P/35) and Exponent software (Stable Micro Systems). The established parameters were test option and mode= measurement of the compression force and hold until the stipulated time; pretest speed = 2.0 mm/s; test speed = 1.7 mm/s; posttest speed = 10.0 mm/s; distance = 10 mm; time = 5 s; trigger-type auto; and force = 0.04903 N. The height of the first force peak is the hardness value measured in  $N$ .

The tests for all formulations were executed at room temperature, and different parameters such as hardness, firmness, cohesiveness, gumminess, springiness, adhesiveness, chewiness, and resilience were analyzed using a force-time graph.

### 3. Thermal Analysis

**3.1. Differential Scanning Calorimetry (DSC).** The ice-melting enthalpy in frozen French bread dough was measured with DSC as follows: 10 mg of the sample was transferred to an aluminum pan. The DSC cell was flushed with nitrogen at 50 mL/min. The sample was cooled to -60°C at a rate of -10°C/min, held for 5 min at -60°C, and heated to 90°C at a rate of 2°C/min. Control measurements with empty pans confirmed that no moisture condensation occurred during the transport of the container into the calorimeter.

**3.2. Thermogravimetric Analysis (TGA).** TGA was conducted on the frozen French bread dough formulations using an analyzer from TA Instruments (Model Q50, New Castle, DE, USA). First, 10 mg of each sample was placed in a platinum pan. Then, it was heated over a range of 25°C-250°C at 10°C/min under an oxidative atmosphere.

**3.3. Scanning Electron Microscopy (SEM).** Before analysis, samples of bread dough were placed on aluminum stubs and sputter-coated with gold. Images were captured with a TESCAN SEM operating at an acceleration voltage of 15 keV. Wheat flour galactomannan and xyloglucan powder were also submitted to the same procedure for further structural review.

**3.4. Statistical Analysis.** The experimental data (mean ± SEM) were compared using one-way ANOVA with a multiple-range Tukey's test at a significance level of  $p < 0.05$  in the STATISTICA 10 software. Linear regression analysis was performed using OriginPro® 8.0 software to compare the differences in the texture parameters hardness, firmness, cohesiveness, gumminess, springiness, adhesiveness, chewiness, and resilience.

### 4. Results and Discussion

**4.1. Hemicellulose Extraction.** In this work, endosperms and cotyledons were manually separated from germ and hull, with yield percentages of  $19.15 \pm 2.98$  for galactomannan and  $8.52 \pm 2.40$  for xyloglucans. Although these results appear inferior to those previously reported [3, 13], we prioritized low protein content because these molecules can impair the rheological behavior of carbohydrates. The measured protein content for *C. pulcherrima* galactomannan was  $1.23 \pm 0.04$ , and *T. indica* xyloglucan was  $7.97 \pm 0.04$ ,

a high protein content in polysaccharides but is reasonable because xyloglucans are used not only as storage but also as a structural polysaccharide in plants. In addition, commercial guar gum can contain more than 10% protein due to industrial processes in which the endosperm is not separated from the hull and cotyledon before extraction [18].

The wheat flour in this work was analyzed. The parameters were compared to Brazilian regulations, which establish limits of 15% ( $w/w$ ) for moisture and 0.8% ( $w/w$ ) for ashes for this wheat flour (type I) [17]. The flour used in this study contained 13.50% water and 0.65% ashes. The lightness ( $L^*$ ) value was 92.52, as close to 100% white as flour which is suitable for bakery goods can be. The gluten index (GI) was 99.10%, which classifies this flour as strong wheat flour ( $GI > 80\%$ ) [19]. The falling number of the wheat flour was 440.00 s, which may reflect a low  $\alpha$ -amylase activity, resulting in reduced bread volume and increased crumbs. During dough fermentation, yeast produces  $CO_2$  and flavor compounds. The gas-forming ability of the enzyme strictly depends on the number of fermentable sugars. The fermentable sugars in wheat flour are insufficient to support yeast growth and need to be enhanced by additional sugars produced from starch by  $\alpha$ - and  $\beta$ -amylase action [20].

**4.2. The Effect of Hemicellulose on Moisture and Water Activity ( $a_w$ ).** The water activity and moisture over 60 days are displayed in Table 2. For the moisture, the HXG sample showed significant differences at 30 and 60 days of frozen storage. However, in terms of the percentages of moisture and water activity, the hemicelluloses did not affect the parameters studied. The amount, physical state, and localization of water are crucial to frozen dough quality [21]. In this work, the hemicelluloses, which are hydrocolloids, did not promote hydrophilic ability during the formation of the wheat dough.

The samples did not present significant differences in  $a_w$  during frozen storage but showed differences between them at time zero. The analyzed samples presented a higher content of open water at time zero. Here, pasta samples made from wheat flour with added galactomannan and xyloglucan retained free water throughout cold storage, which can be explained by this observation.

Water activity is considered an important parameter related to free water, contributing to chemical reactions and microbial development in food. Sharadanant & Khan [22] observed no changes in  $a_w$  in gum-containing masses for up to 16 weeks of storage [23]. A reduction in values in xanthan gum masses and masses with a mixture of guar and xanthan gum has also been observed after 70 days of storage [24]. Water activity in frozen dough formulations containing xanthan gum at the time of freezing presented no significant differences. The guar gum masses showed a substantial reduction in water activity after 70 days of storage.

**4.3. The Effect of Hemicelluloses on Texture, DSC, TGA, and SEM Images.** The results regarding texture are presented in Table 3, showing the values observed for HG, HX, and HXG over 60 days. The fresh dough (T0) formulations with

added galactomannan and xyloglucan (HXG) presented appropriate hardness and firmness values. After 5 days, the HXG sample did not differ from the HX sample. No samples presented a difference after 30 and 60 days of frozen storage. The hardness values of the HC, HG, and HXG samples remained stable throughout cold storage, showing differences only during the freezing process between time zero and at 5 days.

The firmness of the HC and HG samples remained stable over the entire frozen storage time. The firmness of the HXG sample was reduced at 60 days. Other authors who have studied the application of insoluble fiber in bread manufacturing also observed decreased firmness of the food, affirming that the hardening speed is higher in frozen pasta [25]. This was not observed in this study since the use of hemicelluloses produced masses with less firmness after freezing [26]. Adhesiveness did not significantly differ for all formulations at the same time point. Only the formulations containing xyloglucans (HX) presented substantial differences across the storage time.

The elasticity of the HC and HXG samples differ significantly during the study period since freezing the mass and its storage reduces the volume elasticity, which indicates gluten. The HX and HXG samples presented different cohesiveness after 60 days of freezing, although did not result significantly reduced over the time. Other authors have found that cohesiveness was reduced over the storage time of three days, varying from nearly 0.75 to 0.65 [27].

The gumminess and chewiness of the HC, HG, and HXG samples were affected by the freezing process (between time zero and day 5) but remained stable during frozen storage (5, 30, and 60 days). The HX sample always showed a difference. According to Ulzizjargal, Yang, Lin, Chen, & Mau [28], other components in baking tend to change the texture characteristics, including gumminess. Despite having significant differences in the resilience parameter during the frozen storage period, there were no significant differences between the studied samples after 60 days of freezing. The samples did not present substantial differences in all of the texture parameters at the last analyzed time.

DSC was used to analyze the microstructure of the HC dough and the HG, HX, and HXG doughs with added hemicelluloses at time zero and 5, 30, and 60 days of frozen storage (Figures S1–S4). DSC provides an easy way to evaluate the nonfreezable water (NFW) content in foods [29].

The DSC parameters were analyzed to evaluate the impact of added hemicelluloses on the wheat flour used in frozen pasta on the thermal properties during frozen storage. The melting enthalpy ( $\Delta H_m$ ) and solidification enthalpy ( $\Delta H_s$ ) values of the fresh masses (time zero) and frozen storage (5, 30, and 60 days) are shown in Table 4.

Due to their water retention property, hydrocolloids can confer stability to products that undergo freeze-thaw cycles. Also, the adding of hydrocolloids to dough preparations reduces mechanical damage to the gluten network and further improves the quality of frozen dough [30]. In our work, the HC and HXG samples showed the lowest enthalpy of solidification values after 60 days of frozen storage, whereas the HG and HX samples presented the highest benefits at this time.

TABLE 2: Average moisture and water activity values of wheat flour with added *Caesalpinia pulcherrima* galactomannan and *Tamarindus indica* xyloglucan.

Parameters	Batch	Time (days)			
		0	5	30	60
$a_w$	HC	0.967 ± 0.004 <sup>aA</sup>	0.974 ± 0.006 <sup>aA</sup>	0.970 ± 0.004 <sup>aA</sup>	0.975 ± 0.001 <sup>aA</sup>
	HG	0.975 ± 0.002 <sup>bA</sup>	0.972 ± 0.003 <sup>aA</sup>	0.969 ± 0.002 <sup>aA</sup>	0.974 ± 0.003 <sup>aA</sup>
	HX	0.973 ± 0.003 <sup>abA</sup>	0.973 ± 0.002 <sup>aA</sup>	0.973 ± 0.005 <sup>aA</sup>	0.972 ± 0.005 <sup>aA</sup>
	HXG	0.974 ± 0.003 <sup>abA</sup>	0.967 ± 0.006 <sup>aA</sup>	0.972 ± 0.004 <sup>aA</sup>	0.968 ± 0.004 <sup>aA</sup>
Moisture (%)	HC	58.42 ± 0.06 <sup>abA</sup>	58.22 ± 0.05 <sup>AB</sup>	57.73 ± 0.10 <sup>aC</sup>	57.81 ± 0.12 <sup>aC</sup>
	HG	58.28 ± 0.10 <sup>bA</sup>	58.09 ± 0.24 <sup>aAB</sup>	57.58 ± 0.23 <sup>aB</sup>	57.67 ± 0.31 <sup>aB</sup>
	HX	58.37 ± 0.06 <sup>bA</sup>	58.18 ± 0.33 <sup>aA</sup>	57.78 ± 0.10 <sup>aB</sup>	57.89 ± 0.09 <sup>aB</sup>
	HXG	58.63 ± 0.07 <sup>aA</sup>	58.38 ± 0.08 <sup>aAB</sup>	58.18 ± 0.17 <sup>bB</sup>	58.39 ± 0.22 <sup>bAB</sup>

HC: sample control containing only wheat flour. HG: dough prepared with *C. pulcherrima* galactomannan. HX: dough prepared with *T. indica* xyloglucan. HXG: dough prepared with both galactomannan and xyloglucan. Results are expressed as mean ± sd. Equal capital letters in the same line do not present significant differences ( $p < 0.05$ ) comparing the same formulation at different times. Equal lowercase letters in the same column do not present a significant difference ( $p < 0.05$ ) comparing the formulations over the same time.

The freezing process (between time zero and day 5) caused an increase in the solidification enthalpy in the HC, HX, and HXG samples, while the HG sample decreased slightly. HX presented the most stable solidification enthalpy values during the cold storage (times zero, 5, 30, and 60 days).

The HG sample had a significantly higher melting enthalpy than the other samples at time zero. The HX sample had behavior similar to that of HG after 60 days of frozen storage and showed higher enthalpy of fusion values at this time. The HC sample had stable behavior until 30 days and presented a smaller melting enthalpy after 60 days of frozen storage. The HG sample showed a reduction in this parameter between time zero and day 5, an increase between 5 and 30 days, and reduced again between 30 and 60 days of frozen storage. The HX sample showed an increase in the melting enthalpy after the freezing process (between time zero and day 5) and was stable during frozen storage (5, 30, and 60 days). The melting enthalpy of the HXG sample increased after the freezing process and remained stable between 5 and 30 days, but reduced after 60 days of frozen storage. The HX and HXG samples did not show a reduction in the melting enthalpy at 60 days compared to time zero. When the hemicelluloses were added (HG, HX, and HXG), the best consistency conditions of the frozen water state were in the HX and HG samples according to the DSC analysis.

When guar gum has been incorporated into frozen doughs, the enthalpy of melting has tended to decrease after the freezing process, which is in agreement with the results found in this study for galactomannan [24]. The enthalpy of the samples with added xyloglucans increased after freezing. In general, the samples without added guar gum and the sample with 0.036 g/100 g of added guar gum had the largest changes in enthalpy. These samples also presented higher values throughout the cold storage, while the samples with 0.125 g/100 g, 0.214 g/100 g, and 0.250 g/100 g of added guar gum underwent minor changes and presented lower mean values of the enthalpy of melting [24].

No changes were observed in doughs containing xanthan gum after the freezing process (time zero and 0.1 days of storage). In general, the samples without xanthan

gum and 0.036 and 0.125 g/100 g of added xanthan gum showed higher mean enthalpy values, while adding 0.214 and 0.250 g/100 g of xanthan gum showed lower melting enthalpies during frozen storage [24].

Table 5 shows the initial solidification temperatures ( $T_{onsets}$ ) and the initial melting temperatures ( $T_{onsetm}$ ) of the fresh masses (time zero) and over the frozen storage time (time 5, 30, and 60 days).

At 60 days, the HC and HXG samples suffered more from temperature variations in the storage equipment since their initial melting temperatures were the lowest ( $-9.58^\circ\text{C}$  and  $-10.04^\circ\text{C}$ ), while the HG and HX samples presented higher initial melting temperatures ( $-8.98^\circ\text{C}$  and  $-8.17^\circ\text{C}$ ), respectively, showing higher resistance to temperature variations during frozen storage and transport. Table 6 shows the final solidification temperatures ( $T_{endsets}$ ) and final melting temperatures ( $T_{endsetm}$ ) of the fresh doughs (time zero) and over the frozen storage time (5, 30, and 60 days).

The HG sample has higher final freezing temperatures,  $-20.94^\circ\text{C}$  and  $-17.47^\circ\text{C}$  at time zero and 30 days, respectively. At 5 days, the HXG sample had the highest freezing temperature ( $-18.11^\circ\text{C}$ ), and at 60 days, the HX sample had the highest final freezing temperature ( $-18.98^\circ\text{C}$ ). The samples with added hemicellulose generally had more efficient final freezing temperatures.

At time zero, the HG sample thawed at the highest temperature ( $-0.97^\circ\text{C}$ ). All the samples presented similar behavior for the final heat of fusion at 5 days, while the samples with added hemicellulose presented higher  $T_{endsetm}$  temperatures at 30 days. The HG and HX samples showed higher  $T_{endsetm}$  values at 60 days, while the HC and HXG samples showed lower  $T_{endsetm}$ .

The results for the peak solidification temperature ( $T_{peaks}$ ) and peak melting temperature ( $T_{peakm}$ ) parameters of the fresh masses (time zero) and over the frozen storage time (5, 30, and 60 days) are shown in Table 7.

The HG sample showed better freezing efficiency at time zero, with a higher  $T_{peaks}$  value. The samples had similar behavior at 5 days of freezing, whereas samples with added hemicellulose presented better freezing efficiency at 30 days

TABLE 3: Parameters of texture profile analysis.

Parameters	Batch	Time (days)			
		0	5	30	60
Hardness	HC	595.79 ± 131.37 <sup>aA</sup>	184.82 ± 25.47 <sup>aB</sup>	196.11 ± 32.10 <sup>aB</sup>	264.45 ± 58.32 <sup>aB</sup>
	HG	476.99 ± 92.81 <sup>aA</sup>	236.19 ± 31.84 <sup>bB</sup>	178.84 ± 25.00 <sup>aB</sup>	235.40 ± 43.07 <sup>aB</sup>
	HX	615.21 ± 33.76 <sup>aA</sup>	332.44 ± 25.85 <sup>cB</sup>	200.82 ± 16.23 <sup>aC</sup>	279.86 ± 20.82 <sup>aD</sup>
	HXG	780.38 ± 76.53 <sup>bA</sup>	317.72 ± 21.37 <sup>cB</sup>	229.46 ± 53.90 <sup>aB</sup>	243.89 ± 34.24 <sup>aB</sup>
Springiness	HC	653.56 ± 153.41 <sup>aA</sup>	202.64 ± 28.85 <sup>aB</sup>	214.05 ± 35.93 <sup>aB</sup>	287.35 ± 63.87 <sup>aB</sup>
	HG	522.99 ± 105.99 <sup>aA</sup>	257.49 ± 35.47 <sup>bB</sup>	193.17 ± 28.50 <sup>aB</sup>	255.01 ± 47.89 <sup>aB</sup>
	HX	677.20 ± 41.01 <sup>aA</sup>	362.45 ± 30.20 <sup>cB</sup>	215.38 ± 17.50 <sup>aC</sup>	302.32 ± 21.70 <sup>aD</sup>
	HXG	877.43 ± 85.99 <sup>bA</sup>	345.60 ± 25.34 <sup>cA</sup>	247.36 ± 59.19 <sup>aA</sup>	262.00 ± 37.44 <sup>aB</sup>
Adhesiveness	HC	-352.62 ± 40.86 <sup>aA</sup>	-318.08 ± 151.90 <sup>aA</sup>	-378.30 ± 75.87 <sup>aA</sup>	-405.24 ± 55.13 <sup>abA</sup>
	HG	-362.15 ± 119.61 <sup>aA</sup>	-356.86 ± 79.28 <sup>aA</sup>	-331.04 ± 47.61 <sup>aA</sup>	-351.37 ± 63.80 <sup>aA</sup>
	HX	-464.91 ± 35.03 <sup>aA</sup>	-398.15 ± 100.02 <sup>aAB</sup>	-296.43 ± 32.64 <sup>aA</sup>	-443.50 ± 37.39 <sup>aBB</sup>
	HXG	-474.38 ± 57.41 <sup>aA</sup>	-382.63 ± 32.11 <sup>aA</sup>	-216.70 ± 164.21 <sup>aA</sup>	-406.65 ± 21.65 <sup>aA</sup>
Firmness	HC	0.972 ± 0.01 <sup>aA</sup>	0.857 ± 0.124 <sup>aA</sup>	0.909 ± 0.014 <sup>aA</sup>	0.932 ± 0.019 <sup>aA</sup>
	HG	0.966 ± 0.009 <sup>aA</sup>	0.926 ± 0.017 <sup>aB</sup>	0.903 ± 0.008 <sup>aC</sup>	0.934 ± 0.011 <sup>aD</sup>
	HX	0.976 ± 0.002 <sup>aA</sup>	0.949 ± 0.009 <sup>aB</sup>	0.929 ± 0.016 <sup>aC</sup>	0.944 ± 0.006 <sup>aBC</sup>
	HXG	0.976 ± 0.002 <sup>aA</sup>	0.945 ± 0.005 <sup>aA</sup>	0.898 ± 0.111 <sup>aA</sup>	0.923 ± 0.020 <sup>aA</sup>
Cohesiveness	HC	0.866 ± 0.03 <sup>aA</sup>	0.768 ± 0.128 <sup>aA</sup>	0.808 ± 0.016 <sup>aA</sup>	0.841 ± 0.062 <sup>aA</sup>
	HG	0.775 ± 0.068 <sup>aA</sup>	0.789 ± 0.030 <sup>aA</sup>	0.816 ± 0.022 <sup>aA</sup>	0.840 ± 0.037 <sup>aA</sup>
	HX	0.789 ± 0.013 <sup>aA</sup>	0.808 ± 0.021 <sup>aA</sup>	0.813 ± 0.013 <sup>aA</sup>	0.869 ± 0.013 <sup>aB</sup>
	HXG	0.787 ± 0.015 <sup>aA</sup>	0.805 ± 0.029 <sup>aA</sup>	0.779 ± 0.068 <sup>aA</sup>	0.875 ± 0.011 <sup>aB</sup>
Gumminess	HC	562.55 ± 116.52 <sup>abA</sup>	154.59 ± 28.81 <sup>aB</sup>	172.96 ± 29.16 <sup>aB</sup>	240.91 ± 51.53 <sup>aB</sup>
	HG	408.63 ± 99.62 <sup>aA</sup>	202.82 ± 23.01 <sup>bB</sup>	157.66 ± 23.23 <sup>aB</sup>	214.53 ± 42.84 <sup>aB</sup>
	HX	534.55 ± 34.82 <sup>aA</sup>	293.13 ± 28.37 <sup>cB</sup>	175.15 ± 13.15 <sup>aC</sup>	262.77 ± 17.57 <sup>aD</sup>
	HXG	690.71 ± 66.99 <sup>bA</sup>	278.11 ± 15.55 <sup>cB</sup>	194.95 ± 56.02 <sup>aB</sup>	229.62 ± 34.05 <sup>aB</sup>
Chewiness	HC	547.86 ± 117.50 <sup>abA</sup>	134.76 ± 38.19 <sup>aB</sup>	157.38 ± 26.84 <sup>aB</sup>	225.18 ± 52.12 <sup>aB</sup>
	HG	395.53 ± 98.90 <sup>aA</sup>	187.99 ± 23.10 <sup>bB</sup>	142.47 ± 22.04 <sup>aB</sup>	200.81 ± 42.36 <sup>aB</sup>
	HX	521.87 ± 34.82 <sup>abA</sup>	278.02 ± 24.82 <sup>cB</sup>	162.91 ± 13.82 <sup>aC</sup>	248.19 ± 17.96 <sup>aD</sup>
	HXG	674.72 ± 66.51 <sup>bA</sup>	263.07 ± 14.44 <sup>cB</sup>	179.05 ± 63.32 <sup>aB</sup>	212.58 ± 35.34 <sup>aB</sup>
Resilience	HC	0.163 ± 0.040 <sup>aA</sup>	0.114 ± 0.024 <sup>aA</sup>	0.126 ± 0.017 <sup>aA</sup>	0.144 ± 0.032 <sup>aA</sup>
	HG	0.11 ± 0.03 <sup>bA</sup>	0.122 ± 0.020 <sup>aAB</sup>	0.141 ± 0.016 <sup>abAB</sup>	0.147 ± 0.017 <sup>aB</sup>
	HX	0.106 ± 0.006 <sup>bA</sup>	0.130 ± 0.006 <sup>aB</sup>	0.134 ± 0.006 <sup>abB</sup>	0.142 ± 0.011 <sup>aB</sup>
	HXG	0.118 ± 0.010 <sup>abA</sup>	0.124 ± 0.015 <sup>aAB</sup>	0.157 ± 0.021 <sup>bC</sup>	0.151 ± 0.010 <sup>aBC</sup>

HC: sample control containing only wheat flour. HG: dough prepared with galactomannan. HX: dough prepared with xyloglucan. HXG: dough prepared with both galactomannan and xyloglucan. Results are expressed as mean ± sd. Equal capital letters in the same line do not present significant differences ( $p < 0.05$ ) comparing the same formulation at different times. Equal lowercase letters in the same column do not present a significant difference ( $p < 0.05$ ) comparing the formulations over the same time.

since the peak of this process occurred at higher temperatures than the control sample. The HG and HX samples had  $T_{peaks}$  at higher temperatures at 60 days of frozen storage. The added gum samples showed a thawing peak at higher temperatures at all analyzed times, demonstrating resistance to the temperature variations of the frozen storage equipment.

Thermogravimetric analysis (TGA) of the fresh dough samples (HC, HG, HX, and HXG) at time zero and the frozen samples at 5, 30, and 60 days of frozen storage are given in Figures S1–S12 of the Supplementary Material. The microstructures of the HC, HG, HX, and HXG doughs

were analyzed at time zero and 5, 30, and 60 days of frozen storage (Figures S1–S4). Table 8 shows the mass loss of samples (HC, HG, HX, and HXG) of fresh dough (time zero) and frozen samples at 5, 30, and 60 days of frozen storage. The HC and HXG samples showed similar water loss at time zero, indicating that adding the hemicelluloses to the HXG sample was beneficial and prevented more significant water losses. The HG and HX samples contained the individual hemicelluloses and had the highest water loss values. The HX sample had the highest mass loss at 5 days of freezing, while the HXG sample had the lowest loss.

TABLE 4: Melting enthalpy ( $\Delta H_m$ ) and solidifying enthalpy ( $\Delta H_s$ ) of doughs made from wheat flour with added *Caesalpinia pulcherrima* galactomannan and *Tamarindus indica* xyloglucan.

Batch	Parameters (J/g)	Time (days)			
		0	5	30	60
HC	$\Delta H_s$	43.05	50.12	47.01	34.29
	$\Delta H_m$	41.86	45.57	44.96	33.72
HG	$\Delta H_s$	52.51	49.84	60.05	50.81
	$\Delta H_m$	53.18	46.61	54.98	49.50
HX	$\Delta H_s$	41.39	55.56	52.01	51.25
	$\Delta H_m$	39.74	54.17	50.99	52.31
HXG	$\Delta H_s$	32.08	51.50	50.94	30.59
	$\Delta H_m$	27.95	49.96	51.29	32.02

HC: sample control containing only wheat flour. HG: dough prepared with galactomannan. HX: dough prepared with xyloglucan. HXG: dough prepared with both galactomannan and xyloglucan.

TABLE 5: Initial solidification temperature ( $T_{onset_s}$ ) and initial melting temperature ( $T_{onset_m}$ ) of the pasta made with wheat flour containing added *Caesalpinia pulcherrima* galactomannan and *Tamarindus indica* xyloglucan.

Batch	Parameter ( $^{\circ}\text{C}$ )	Time (days)			
		0	5	30	60
HC	$T_{onset_s}$	-14.56	-13.13	-12.85	-14.06
	$T_{onset_m}$	-8.56	-7.88	-8.07	-10.04
HG	$T_{onset_s}$	-12.22	-12.83	-11.54	-11.48
	$T_{onset_m}$	-7.13	-8.21	-8.45	-8.98
HX	$T_{onset_s}$	-13.95	-8.28	-12.03	-12.84
	$T_{onset_m}$	-8.79	-7.64	-7.84	-8.17
HXG	$T_{onset_s}$	-13.81	-13.03	-11.66	-14.45
	$T_{onset_m}$	-8.86	-8.31	-8.20	-9.58

HC: sample control containing only wheat flour. HG: dough prepared with galactomannan. HX: dough prepared with xyloglucan. HXG: dough prepared with both galactomannan and xyloglucan.

TABLE 6: Final solidification temperature ( $T_{endset_s}$ ) and final melting temperature ( $T_{endset_m}$ ) of the doughs made with wheat flour with added *Caesalpinia pulcherrima* galactomannan and *Tamarindus indica* xyloglucan.

Batch	Parameters ( $^{\circ}\text{C}$ )	Time (days)			
		0	5	30	60
HC	$T_{endset_s}$	-21.05	-19.39	-18.96	-20.12
	$T_{endset_m}$	-2.66	-2.49	-2.33	-2.99
HG	$T_{endset_s}$	-20.94	-19.69	-17.47	-19.36
	$T_{endset_m}$	-0.97	-2.11	-1.76	-1.34
HX	$T_{endset_s}$	-21.85	-18.18	-18.56	-18.98
	$T_{endset_m}$	-1.64	-2.28	-1.57	-1.86
HXG	$T_{endset_s}$	-22.45	-18.11	-18.70	-20.54
	$T_{endset_m}$	-2.11	-2.47	-1.96	-2.87

HC: sample control containing only wheat flour. HG: dough prepared with galactomannan. HX: dough prepared with xyloglucan. HXG: dough prepared with both galactomannan and xyloglucan.

The HXG sample showed the lowest water loss up to 30 days of frozen storage, possibly indicating synergism between the hemicelluloses added to the mass that contributed to the higher water retention, which promotes the stability of the gluten. Hence, the HXG sample obtained the best performance until 30 days of frozen storage.

The temperature at which the peak mass loss event occurred was verified by the mass derivatives as a function of the temperature of the fresh mass (time zero) and the frozen samples at 5, 30, and 60 days of frozen storage (Table 7). The samples showed successive increases in the peak temperature of the mass loss over the freezing time (5, 30, and

TABLE 7: Peak solidification temperature ( $T_{peak_s}$ ) and peak melting temperature ( $T_{peak_m}$ ) of doughs made from wheat flour with added *Caesalpinia pulcherrima* galactomannan and *Tamarindus indica* xyloglucan.

Batch	Parameters (°C)	Time (days)			
		0	5	30	60
HC	$T_{peak_s}$	-15.44	-13.93	-13.84	-15.27
	$T_{peak_m}$	-3.66	-3.52	-3.24	-4.26
HG	$T_{peak_s}$	-13.25	-13.65	-12.04	-12.93
	$T_{peak_m}$	-2.39	-3.00	-2.70	-2.61
HX	$T_{peak_s}$	-15.50	-13.37	-12.95	-13.80
	$T_{peak_m}$	-2.78	-3.30	-2.74	-2.89
HXG	$T_{peak_s}$	-15.81	-13.74	-12.33	-16.20
	$T_{peak_m}$	-3.20	-3.34	-2.98	-3.87

HC: sample control containing only wheat flour. HG: dough prepared with galactomannan. HX: dough prepared with xyloglucan. HXG: dough prepared with both galactomannan and xyloglucan.

TABLE 8: Weight loss and peak temperature in the thermogravimetric analysis of dough made with wheat flour containing added *Caesalpinia pulcherrima* galactomannan and *Tamarindus indica* xyloglucan.

	Batch	Time (days)			
		0	5	30	60
Weight loss (%)	HC	31.25	34.95	39.70	36.27
	HG	33.85	35.90	40.39	39.65
	HX	34.39	37.17	39.58	40.99
	HGX	31.39	32.54	37.49	40.42
Peak temperature of weight loss (°C)	HC	110.90	108.52	129.39	142.25
	HG	108.52	107.94	128.18	137.38
	HX	110.94	112.20	124.50	155.15
	HGX	109.78	119.56	135.54	137.96

HC: sample control containing only wheat flour. HG: dough prepared with galactomannan. HX: dough prepared with xyloglucan. HXG: dough prepared with both galactomannan and xyloglucan.

60 days). The HX sample had the highest peak temperature during the 60 days of frozen storage, while the HXG sample presented the highest results at 5 and 30 days.

At the peak temperatures, the HXG sample was the most stable for storage times up to 30 days. Fresh HXG dough presented similar behavior to the HC sample with lower mass losses. The HXG dough had the most moderate mass loss among the four samples after 5 days of frozen storage, followed by the HC sample, while the HX and HG samples presented higher losses. The smallest mass loss at 30 days of frozen storage was observed in the HXG sample, while the lowest mass loss at 60 days of frozen storage was measured in the HC sample.

Figure 1 shows the SEM images of the main ingredients studied: wheat flour, galactomannan, and xyloglucan. These images were collected separately before ingredients were added to the dough. As expected, the starch gave the wheat flour a dispersed and nonuniform surface. Xyloglucan particles of varying sizes, shapes, roughnesses, and possible aggregation were viewed in the SEM image. Galactomannan appeared more fibrous than wheat flour and xyloglucan, but also had more spherical particles. The fibers were not smooth and appeared to be formed of microfibrils.

Figure 2 shows the SEM images of the HC, HG, HX, and HXG preparations, presenting their microstructure over 60 days of storage under freezing conditions. From time zero to 30 days, the images show that the cohesiveness of the material did not differ significantly among treatments, while significant differences were observed in HX and HXG at 60 days after formulation. This effect might be attributed to the cohesiveness values found once this parameter is intrinsically related to the deformation before rupture.

When comparing the samples which have been frozen for the same amount of time (5 days), greater cohesion can be observed in the additive samples (HG, HX, and HXG) than in the control sample (HC). The HC sample has more void spaces. The HC sample was less cohesive, while the HXG sample was more cohesive at 5 days compared to that of time zero. The HX sample presented similar characteristics at time zero and day 5, while the HG sample was more uniform at 5 days compared with time zero. All samples presented fractures caused by ice crystals or sample dryness. The HXG sample presented the best appearance with more discrete and narrower fractures. It also showed less wear on the material after 60 days of frozen storage, which corroborates the moisture analysis in which this sample presented better results.



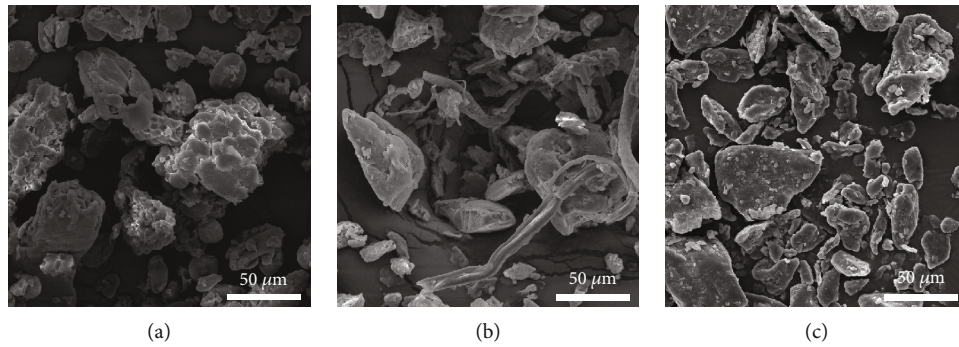


FIGURE 1: Scanning electron microscopy (SEM) images of (a) wheat flour, (b) galactomannan powder, and (c) xyloglucan powder before addition to dough preparations.

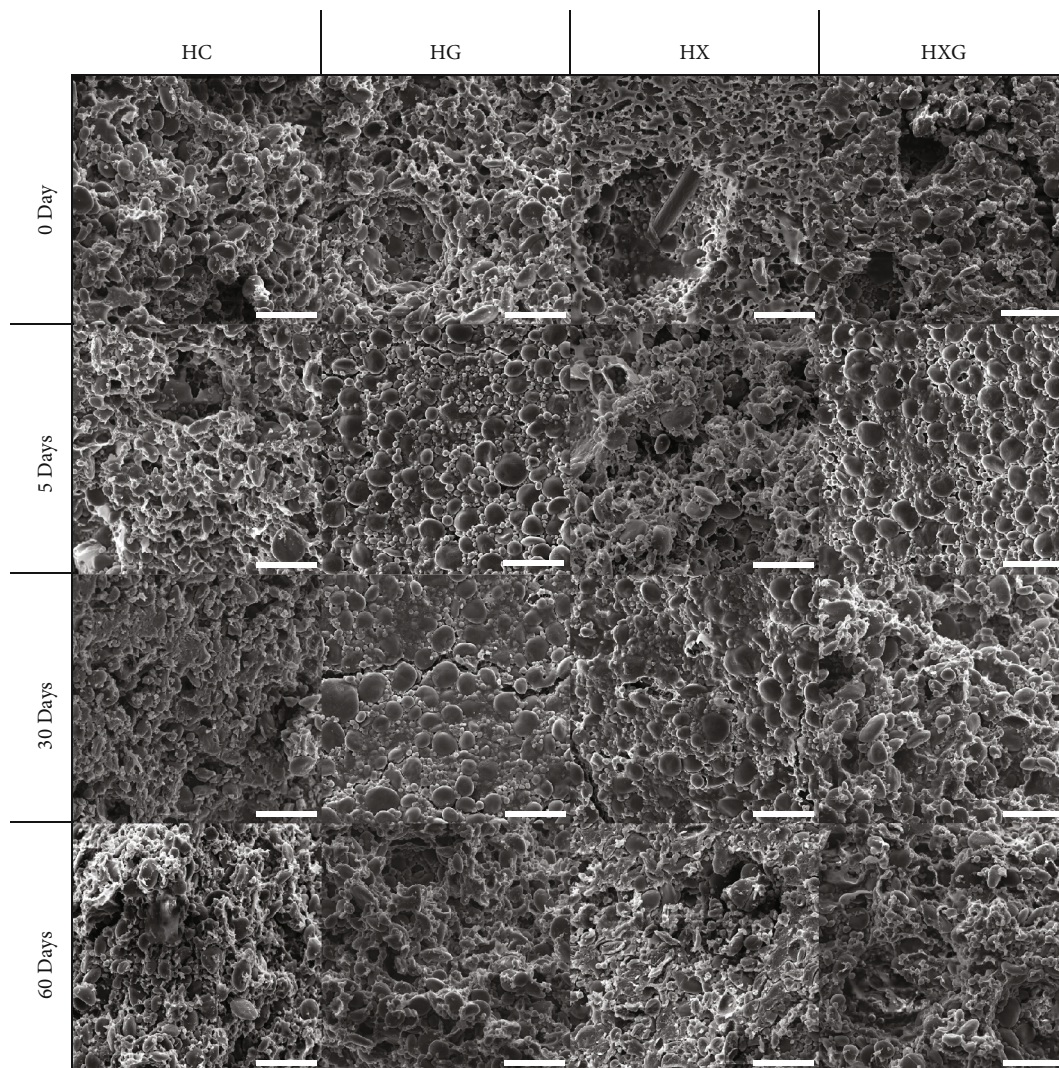


FIGURE 2: SEM images of cross-sections of samples of doughs collected from 0 to 60 days of frozen storage. HC: sample control containing only wheat flour. HG: dough prepared containing galactomannan. HX: dough prepared containing xyloglucan. HXG: dough prepared containing both galactomannan and xyloglucan. Scale bar = 50 μm.

## 5. Conclusion

Adding galactomannan and xyloglucan to frozen doughs reduces the damage caused by cold storage, as samples with added hemicellulose presented better stability for water activity. A combination of hemicelluloses provided higher moisture retention and less wear and tear and fractures over the shelf life. Adding vegetable hemicelluloses to doughs does not interfere with the texture of frozen samples at the end of their shelf life, preserving the preparation at  $-18^{\circ}\text{C}$  for up to 60 days. Applying these hemicelluloses in frozen pasta technology is suggested since it improves essential parameters in the technological quality of bread and pasta during freezing.

## Data Availability

The authors declare that all data supporting the findings of this study are available within the article and its supplementary information files.

## Conflicts of Interest

The authors declare that they have no conflict of interest.

## Authors' Contributions

Luísa Helena Ellery Mourão was responsible for the investigation, formal analysis, and writing—original draft. Francisco Rogênio da Silva Mendes was responsible for the formal analysis and writing—review and editing. Felipe Domingos de Sousa was responsible for the methodology and writing—review and editing. Ana Cristina de Oliveira Monteiro Moreira obtained funding for the study. Stella Regina Arcanjo Medeiros was responsible for the formal analysis and writing—review and editing. Maria do Socorro Rocha Bastos was responsible for the resources and methodology. Gilberto Dantas Saraiva was responsible for the formal analysis. Hélcio Silva dos Santos was responsible for the formal analysis. Alexandre Magno Rodrigues Teixeira was responsible for the formal analysis. Renato de Azevedo Moreira was responsible for supervising and obtaining funding for the study.

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## Supplementary Materials

Figure S1: differential scanning calorimetry (DSC) of control sample (HC) at zero-time day of storage under freezing. Figure S2: differential scanning calorimetry (DSC) of control sample (HC) at 5-time days of storage under freezing. Figure S3: differential scanning calorimetry (DSC) of control sam-

ple (HC) at 30-time days of storage under freezing. Figure S4: differential scanning calorimetry (DSC) of control sample (HC) at 60-time days of storage under freezing. Figure S5: differential scanning calorimetry (DSC) of galactomannan sample (HG) at zero-time day of storage under freezing. Figure S6: differential scanning calorimetry (DSC) of galactomannan sample (HG) at 5-time days of storage under freezing. Figure S7: differential scanning calorimetry (DSC) of galactomannan sample (HG) at 30-time days of storage under freezing. Figure S8: differential scanning calorimetry (DSC) of galactomannan sample (HG) at 60-time days of storage under freezing. Figure S9: Thermogravimetric analysis (TGA) of the fresh dough samples (HC, HG, HX, and HXG) at zero-time day of storage under freezing. Figure S10: Thermogravimetric analysis (TGA) of the fresh dough samples (HC, HG, HX, and HXG) at 5-time days of storage under freezing. Figure S11: Thermogravimetric analysis (TGA) of the fresh dough samples (HC, HG, HX, and HXG) at 30-time days of storage under freezing. Figure S12: Thermogravimetric analysis (TGA) of the fresh dough samples (HC, HG, HX, and HXG) at 60-time days of storage under freezing. (*Supplementary Materials*)

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