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A Comparative Analysis of Partial Replacement of Yeast with CO_2 Gas Hydrates as Leavening Agents in Baking of Wheat Bread

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Abstract: The aim of this study is to examine how CO_2 gas hydrates (CO_2 GH) are used in baking, notably in the creation of wheat bread, as a leavening agent. CO_2 GH were produced with the use of food grade amino acids called promoters. The article emphasizes an interesting approach by using a hybrid combination of yeast and CO_2 GH. The current study is based on a comparative evaluation of leavening agents in bread with (1) GH with promoters (20–70% GH) and (2) GH with promoters + yeast as a partial replacement (25–75%). The results obtained are then also compared with those of standard yeast bread along with the effects on wheat bread quality parameters such as volume, specific volume, hardness, moisture content, baking loss, and pore size. The GH bread with promoters had baking losses that were equivalent to the quality of traditional yeast bread. The breads with 60% and 70% GH with promoters had the best outcomes when compared with standard yeast bread. When a combination of CO_2 GH with promoters (20–70%) and yeast (25–75%) was used in the study, the best combinations were obtained with 70% GH + 50% yeast and 70% GH + 75% yeast. Additionally, this article clarifies some potential future uses for CO_2 GH as leavening agents in the bread industry, with a discussion of potential limitations as well as the cost efficiency of the developed technology. This information could help in the creation of new methods and standards for better CO_2 GH selection for usage in other bakery goods.

Keywords: bread; CO_2 gas hydrates; yeast; leavening; promoters; amino acids

Citation: Srivastava, S.; Kollemparembil, A.M.; Zettel, V.; Claßen, T.; Mobarak, M.; Gatternig, B.; Delgado, A.; Jekle, M.; Hitzmann, B. A Comparative Analysis of Partial Replacement of Yeast with CO_2 Gas Hydrates as Leavening Agents in Baking of Wheat Bread. *Processes* **2023**, *11*, 653. https://doi.org/10.3390/pr11030653

Academic Editor: Chi-Fai Chau

Received: 3 January 2023
Revised: 17 February 2023
Accepted: 20 February 2023
Published: 21 February 2023

1. Introduction

When water and low molecular weight gases are subjected to low temperature and high pressure conditions, gas hydrates (GH) are produced. In GH, van der Waal forces connect the host and guest molecules [1,2]. Nitrogen, carbon dioxide, propane, and ethane are the most commonly employed guest molecules for GH [3,4]. A typical gas hydrate structure has around 85% water molecules, which are bound together by hydrogen bonds to create cages that confine the guest molecules [4,5]. Gas hydrates have qualities that are similar to those of ice due to their high water content. The main differences between hydrates and ice are in their mechanical and thermal characteristics. Hydrate structures are somewhat stronger than ice because the rate of water diffusion in hydrates is lower [6]. Furthermore, compared with ice, gas hydrates have poorer thermal conductivity and a larger heat capacity [5,7]. Gas hydrate formation is typically enhanced by chemical and mechanical approaches [1]. While the chemical technique is used to aid hydrate formation under milder circumstances, boost the formation rate and gas absorption, and improve hydrate selectivity, the mechanical approach seeks to increase the contact area and mass transfer between water and gas [8–10]. However, the mechanical approach is quite expensive because it requires expensive machinery and a lot of energy to produce electricity. Industrial manufacturing does not have great economic advantages, and thus the chemical technique is believed to be the one that will be used the most frequently [11,12]. In large-scale applications, in order to enhance gas storage density, accelerate the formation rate, and minimize the induction time of CO_2 hydrate, either thermodynamic hydrate promoters or kinetic hydrate promoters are used [4]. Thermodynamic hydrate promoters are used to alter the hydrate phase boundary conditions to higher temperatures and lower pressures, while kinetic hydrate promoters are used to improve the hydrate induction time, formation rate, and gas/water absorption during the hydrate formation process [4,7]. Commonly used thermodynamic hydrate promoters include acetone and tetrahydrofuran, while kinetic hydrate promoters include sodium dodecyl sulfate, surfactants, and nanoparticles [13–15]. The various aforementioned hydrate promoters are synthetic in nature, and they are either required in extremely high concentrations to be effective or are dangerous and pose a safety concern. Therefore, researchers are now seeking greener, biodegradable, and benign chemicals that might possibly replace existing conventional promoters. Over the last decade, amino acids have grown in importance as promoters in gas hydrate research, and they have the ability to interact with water electrostatically. Most significantly, they are biodegradable, food grade, water soluble, and environmentally benign, and they can be acquired in bulk at a reasonable price [6,8,9].

In many nations, bread, historically a fermented food, is considered one of the staple foods. Nowadays, several leavening agents are used to make bread [16]. The major source of leavening in bread is provided by carbon dioxide. Yeast is one of the major ingredients commonly used in bakery units for the production of bread. It is essential in the bread making process because it metabolizes carbohydrates and produces carbon dioxide (CO_2) and traces of ethanol [17]. During the fermentation of the bread by the yeast, CO_2 is produced in the dough at the mixing, proofing, and baking stages [18,19]. Thus, the presence of yeast gives bread a slow, consistent rise during baking, particularly enhancing the flavor of the bread as well [20,21]. Yeast leavening does have a few disadvantages, however. It takes a while to get started because it takes yeast a while to ferment before releasing enough gas to force the dough to expand to the right size. Typical industrial processes for creating dough require 15–20 min to mix, 1 h to ferment, 15–25 min to proof, and 50–60 min to achieve final proofing, for a total processing time of 5–5.5 h [17,20]. Over-proofing is another prevalent issue in the baking sector. Maltose produced by damaged starch hydrolysis is often still being digested by yeast cells during over-proofing. Over-proofing has a deleterious influence on the dough's rheological and organoleptic properties, resulting in low-quality final loaves [22,23]. Furthermore, the dough storage space must be large enough to accommodate yeast leavening processes. Because fermentation demands exact temperature and humidity control, this can be costly for bakeries. Eliminating fermentation and cutting proofing times would boost bread production. When yeast is excluded from the bread, some bakeries prefer to use chemical leavening agents instead to reduce time requirements [24,25]. However, these chemical leavening agents such as sodium bicarbonate or ammonium bicarbonate have some major health effects on humans [26,27]. During baking, chemical leavening agents produce carcinogenic chemicals such as acrylamide, which are hazardous to human health [28,29].

This study proposes a novel method of leavening via CO_2 gas hydrates (GH) for the production of wheat bread. This method eliminates the time-limited fermentation needs of yeast and permits the continuous manufacture of ready-to-bake leavened wheat dough by substituting CO_2 GH as the leavening agent. The goal of this study was to create wheat bread leavened with CO_2 GH with promoters (20–70%) that was comparable to standard yeast bread. Also, a study was conducted using a combination of CO_2 GH with promoters (20–70%) and yeast in different percentages (25–75%) to see what difference it would make when yeast and GH are added together in a dough. Finally, the GH bread with promoters and partial replacement with yeast (25–75%) was measured for specific volume, volume, moisture content, baking loss, and hardness. Wheat bread dough may be produced in less than an hour when CO_2 GH with promoters are introduced to the dough preparation process. This makes it possible to produce consistently without using up expensive storage space as is necessary for traditional dough fermentation.

2. Materials and Methods

2.1. Production of CO_2 GH with Promoters

The CO_2 GH were produced with optimized promoters, a combination of food grade amino acids (methionine + leucine + lecithin), in a reactor set up at Process Analytics and Cereal Science Department, University of Hohenheim, Germany, in collaboration with installation support from the Institute of Fluid Mechanics (LSTME), FAU Erlangen, Germany [30,31]. The addition of promoters was carried out to enhance the absorption capacity of CO_2 gas during the production of GH in each cycle. By adding 500 mL of distilled water to the reactor vessel that was attached to the CO_2 gas cylinder at an ideal pressure of 37 bars and a low temperature of 0.5 °C, CO_2 GH with promoters were created after four hours [30,31]) and were stored at −20 °C until further use for the bread baking process.

2.2. Process Description of Standard and GH Bread with Promoters

The standard wheat bread was made with dry yeast (3 g), wheat flour of type 550 (297 g) from Rettenmeier Mühle GmbH company, 6 g of sugar, 6 g of salt, and 161.8 g of water. The Farinograph-AT (Brabender GmbH & Co. KG, Duisburg, Germany) was used to calculate how much water is needed to make bread. The amount of CO_2 GH varied from 20–70% per amount of flour for the GH bread. Another set of experiments was performed with a comparative analysis between GH bread (20–70%) with promoters and GH bread with promoters partially replaced with yeast (25–75%). The percentage of yeast added to the GH bread with promoters was determined with respect to the standard bread.

Each 10 g of GH was found to have 2.5 g of CO_2 and 7.5 g of water. To prevent soft dough, the amount of water in the GH was deducted from the total amount needed for kneading. All dry ingredients were weighed together to make the GH bread with promoters, and water was weighed separately. A slight modification in the methodology was made to eliminate the proofing stage for the bread dough made with GH (20–70%). The farinograph temperature was set to 42 °C before mixing all the dry ingredients in the farinograph and the GH with promoters. This step was necessary as the addition of GH lowers the temperature of the dough, and the low temperature of the GH dough can be overcome by this modified step, thereby making it suitable for baking GH bread with promoters. Then, after thoroughly combining all the dry ingredients in the farinograph, the appropriate amounts of water and GH (20–70%) were added, and the mixture was kneaded for 4 min. The final dough temperature at the end of mixing the ingredients ranged from 34 to 36 °C for all the combinations (20–70% GH/GH+ Yeast). The set of experiments with GH bread (20–70%) with promoters and partial replacement with yeast (25–75%) also had similar steps to those mentioned above, but an additional proofing stage was applied due to the presence of yeast. The proofing time for the recipes containing yeast was 10 min. Finally, the standard bread, GH bread with promoters, and GH bread with promoters and partial replacement with yeast were baked at 240 °C in a baking oven (Piccolo, Wachtel GmbH) for 22 min with 12 s of initial steaming.

2.3. Measurement of the Characteristics of GH Bread with Promoters

The characteristics of GH bread (20–70%) with promoters and partial replacement with yeast (25–75%) were measured and then compared with the standard yeast bread. Each characteristic was measured in triplicate. The statistical analysis was performed using the Microsoft Excel add-on function XLSTAT, 2016. The Fischer test and ANOVA were used to identify the significant difference between the samples at 95% confidence interval and a significance level of $\alpha = 0.05$. Different bread characteristics measured are discussed below.

2.3.1. Baking Loss

The baking loss was computed by Equation (1).

$$\text{Baking loss (\%)} = (1 - \text{bread wt./dough wt.}) \times 100 \quad (1)$$

2.3.2. Moisture Analysis

The moisture content was measured for the standard bread, GH bread (20–70%) with promoters, and partial replacement with yeast (25–75%) with an infrared moisture analyzer (Kern and Sohn GmbH, Balingen-Frommern, Germany) [32].

2.3.3. Volume Analysis

A volume analyzer (Stable Microsystems, Vienna Court, United Kingdom, VolScan Profiler 600) was used to determine the volume of the standard bread, GH bread (20–70%) with promoters, and the GH bread with promoters and partial replacement with yeast (25–75%). Prior to the measurement of the bread samples, a zero-height calibration was completed. For the volume study of bread, a three-pin stage assembly with a laser focus was employed [32].

2.3.4. Pore Analysis

A pore scanner (Hp scan jet 5590, Düsseldorf, Germany) coupled to built-in software Gebäck analysis version 1.4 with Oracle Virtual Tool Box 6.1 was used to determine the number and size of the pores.

2.3.5. Texture Analysis

Before the texture analyzer measurement, the bread was cut into 27 mm thick slices using a bread slicing machine (ADE Panis 250 model, ADE Germany, Hamburg, Germany GmbH). Using a texture profile analyzer, the hardness (N) of the bread was measured (TA-XT2, Stable Microsystems) with a P/36R 36 mm cylindrical probe performing the two-byte test [32,33].

3. Results

3.1. GH Bread Appearance and Characteristics

Figure 1 shows images of standard bread and GH bread with promoters (20–70%). When comparing the GH bread with promoters (20–70%) with the standard bread, the 70% GH obtained the best appearance, followed by 60%, 40%, and 20%. However, the general appearance of the bread made with 20% and 40% GH with promoters was not very good. Additionally, less browning was seen on the GH bread with promoters compared with the conventional bread. Due to the formation of secondary metabolites, yeast influences how the color of the bread will turn out. According to Mohamud [18] and Purlis [24], the production of secondary metabolites through various metabolic pathways by non-enzymatic chemical reactions such as Maillard and caramelization results in the production of brown-colored compounds during baking [32–34]. Figure 2 shows images of GH bread with promoters (20%, 40%, 60%, and 70% GH) along with partial replacement (yeast 25%, 50%, and 70%). It was observed that with an increase in the percentage of GH from 20% to 40% or 60% to 70%, the physical appearance of the bread was positively affected. Also, when the combination of GH (20–40% or 60–70%) along with yeast was added in different amounts (25–75%), there was an increase in the browning of the bread when the amount of yeast was increased from 25% to 75%. Also, the recipe with GH had 6% sugar without any yeast, so the sucrose present in the dough would eventually break down to glucose and fructose. However, the amount of sugar added in the GH recipe was not sufficient to make the Maillard reaction work, due to the absence of yeast. Moreover, some of the preliminary trials performed during the study also focused on increasing the amount of sugar in the GH recipe to achieve a browning effect comparable to the standard yeast bread. It was found that when the amount of sugar was increased substantially, the amount of browning was increased due to the activation of the Maillard reaction. However, this research study was aiming for a healthier product, so the idea of increasing the browning with the addition of more sugar was undesirable and the amount of sugar was thus fixed to 6 g, which was equivalent to the standard recipe. The results of application of yeast along with GH with promoters as a leavening agent in bread further validated that somehow yeast plays a vital role in the browning of the bread.

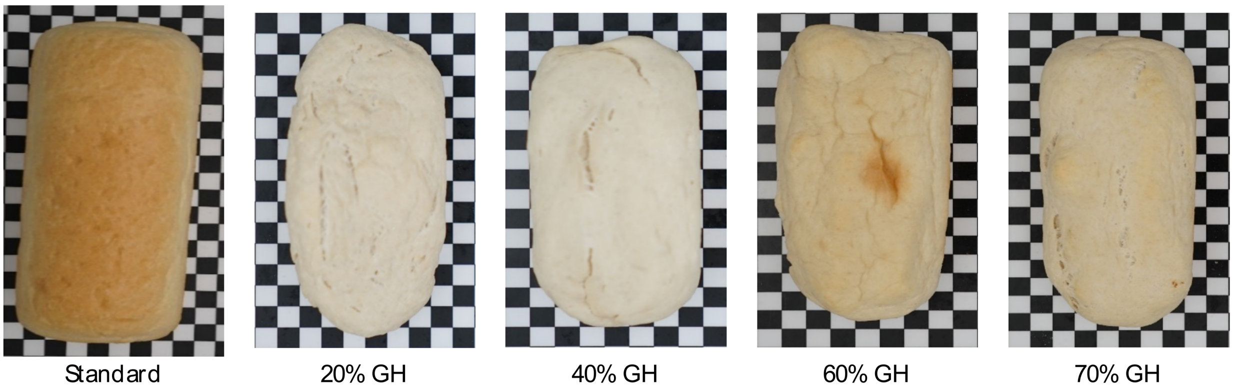

Figure 1. Pictures of standard and GH breads with promoters (20–70%). The pictures show the variation in the bread prepared with GH when promoters are used in different percentages and a comparative evaluation of the color on the crust of the bread with respect to standard bread.

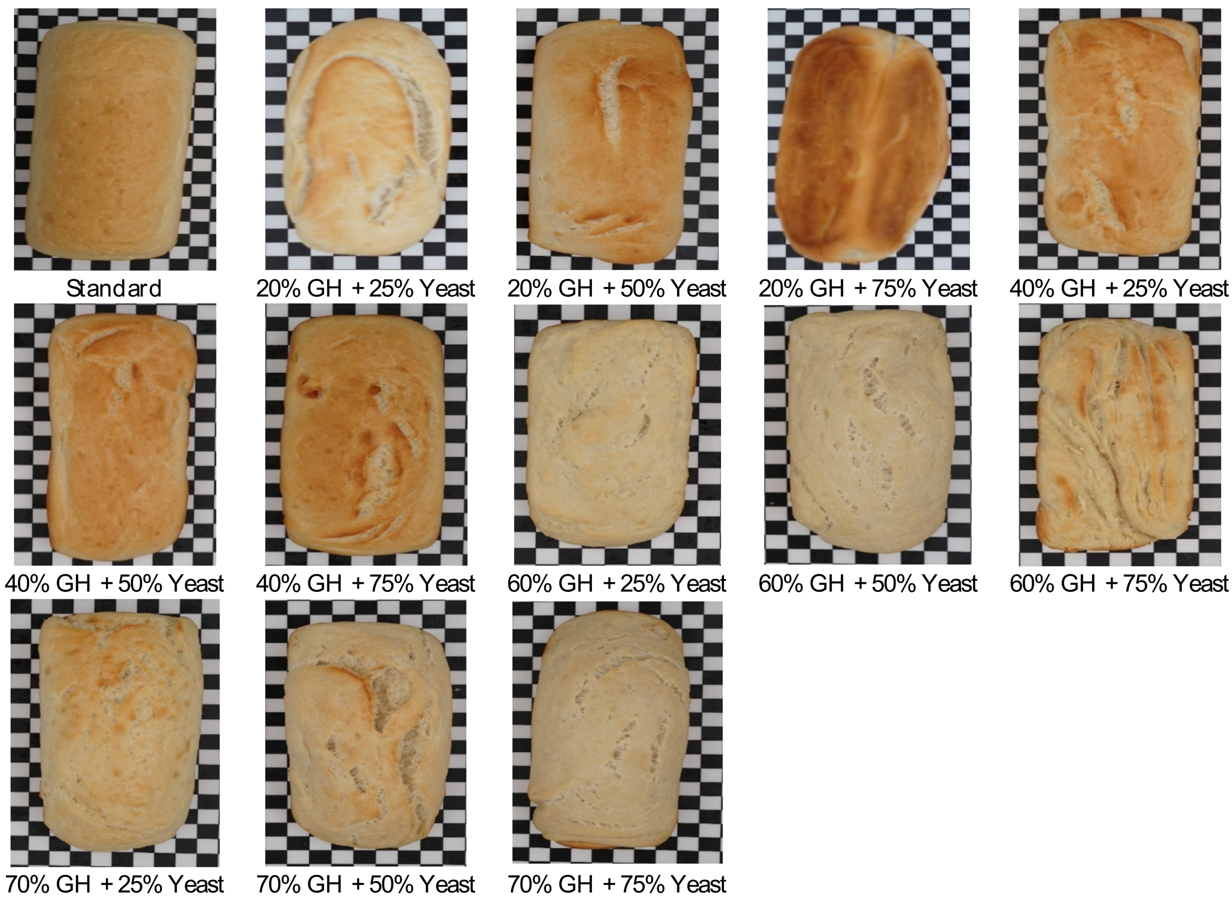

Figure 2. Pictures of GH bread with promoters (20%, 40%, 60%, and 70% GH) and partial replacement (yeast 25%, 50%, and 75%). Different percentages of yeast along with different percentages of GH were used to prepare the bread. It was found that application of yeast along with GH increased the volume of the bread.

Table 1 compares the qualities of normal bread and GH bread with promoters (20–70%). In comparison to the traditional yeast bread, all of the GH bread with promoters had a higher moisture content (mc). Standard yeast bread had a mc of 38%; however, bread made with various GH and promoter (20–70%) percentages had a variable mc ranging from 39% to 44%. The typical yeast bread had a 13% baking loss. The mc loss % of yeast bread could be relatively explained by the proofing stage of yeast bread, which causes some water evaporation compared with the GH bread. The baking loss values in the GH bread with promoters, however, varied from 6.5% to 8.3%. The 70% GH bread (8.3%) had the highest baking loss, which was comparable to the normal yeast bread, demonstrating a reasonable baking loss compared with regular yeast bread. The standard yeast bread volume recorded was 1193 mL; however, in the instance of GH bread with promoters, the volume lay in the range of 596–688 mL. The volume characteristics of 60% and 70% GH bread were found to be 655 mL and 688 mL, respectively. Also, it was found that with the increase in amount of GH percentage from 20% to 70%, there was an increase in the volume of the GH bread due to the addition of more CO_2 from the GH. However, the volume was somewhat less than that of regular bread (Table 1). Additionally, it was shown that the specific volumes of 60% (1.6 mL/g) and 70% (1.9 mL/g) GH bread were equivalent to the typical yeast bread (2.8 mL/g), suggesting that GH with promoters might be utilized as a substitute for yeast as a leavening agent. A higher *p*-value of 0.22 was obtained, which was non-significant, as the computed *p*-value was higher than the alpha of 0.05 significance level. Table 2 shows the characteristics of the standard bread versus GH bread with promoters (20–70%) and partial replacement with yeast (25–75%). The mc of all the GH bread with promoters and partial replacement with yeast was somewhat greater than the standard yeast bread. Standard yeast bread had a mc of 38%, but loaves made with varying amounts of GH and promoters (20–70%) in addition to yeast (25–75%) varied in mc from 35% to 46%. The baking loss for the standard yeast bread was 13%. In the GH bread with promoters and partial replacement with yeast, a baking loss of 7–11% was observed. Also, it was observed that baking loss values were lower for the GH breads with promoters and partial replacement with yeast. This fact might be attributed to the higher moisture content of the GH breads with promoters. Since the amount of water removed during baking was less in the GH bread, less baking loss was observed [35]. The standard yeast bread volume recorded was 1193 mL, while in the case of GH bread with promoters and partial replacement with yeast the volume lay in the range of 749–1052 mL. The best volume characteristics were obtained with 60% GH +75% yeast (956 mL) and 70% GH + 75% yeast (1052 mL) GH bread with promoters and partial replacement. Also, it was found that with the increase in amount of GH percentage from 20% to 70% and the percentage of yeast from 25% to 75%, there was an increase in the volume of the GH bread with promoters due to the addition of more CO_2 from the GH and the yeast. Also, the specific volumes of 70% GH + 50% yeast (1.9 mL/g) and 70% + 75% yeast (2.4 mL/g) GH bread were discovered to be non-significantly different (*p*-value of 0.18) from the standard yeast bread (2.8 mL/g). Therefore, GH with promoters are capable of being used as a substitute for yeast as a leavening agent. However, there is room for development and further thought regarding the bread's volume properties. The yeast metabolism and carbon dioxide production [16] during fermentation are the key factors that affect the volume of each loaf of bread and the bread's aerated cell structure [17,32]. In the GH bread with promoters, the amount of CO_2 incorporated in the dough was lower when a lower amount of the GH was used, while when the concentration of the GH was increased, the volume characteristics of the bread were significantly affected. Also, when a combination of yeast and GH was used, a sufficient amount of CO_2 was produced, which favored the improvement of the bread's overall characteristics. Therefore, a dough's ability to incorporate gas hydrates is crucial for assessing the bread's quality, particularly when GH are used to make the bread. Greater gas hydrate incorporation results in smaller gas cells, which are distributed more evenly, and a greater specific volume is observed [36–38]. Furthermore, variation in the bread volume was visible in the GH bread with promoters due to the fact that flour components are converted into metabolites by enzymes, and yeast present in the flour affects the quality of bread during the fermentation of the dough [39].

Table 1. The characteristics of the standard bread versus GH bread with promoters (20–70%).

	Standard Bread	% of GH			
		20	40	60	70
Moisture (%)	38.0 ± 2.1 ^{a}	41.5 ± 1.7 ^{a}	39.4 ± 1.4 ^{a}	42.3 ± 1.07 ^{a}	44.0 ± 0.4 ^{a}
Baking loss (%)	13.1 ± 2.4 ^{c}	6.5 ± 1.0 ^{c}	7.4 ± 2.0 ^{c}	7.8 ± 3.74 ^{c}	8.3 ± 0.2 ^{c}
Volume (mL)	1193.9 ± 5.5 ^{d}	596.6 ± 11.7 ^{e}	640.6 ± 23.0 ^{e}	655.8 ± 12.2 ^{e}	688.5 ± 17.8 ^{f}
Specific Volume (mL/g)	2.8 ± 1.2 ^{g}	1.1 ± 0.0 ^{g}	1.2 ± 0.4 ^{g}	1.6 ± 0.0 ^{g}	1.9 ± 0.0 ^{g}

Different letters in subscript show they are significantly different from each other.

Table 2. The characteristics of the standard bread versus GH bread with promoters (20–70%) and partial replacement with yeast (25–75%).

	Std Bread	% of GH											
		20 + 25% Yeast	20 + 50% Yeast	20 + 75% Yeast	40 + 25% Yeast	40 + 50% Yeast	40 + 75% Yeast	60 + 25% Yeast	60 + 50% Yeast	60 + 75% Yeast	70 + 25% Yeast	70 + 50% Yeast	70 + 75% Yeast
Moisture (%)	38.0 ± 2.1 ^{a}	41.9 ± 2.4 ^{a}	35.6 ± 3.3 ^{a}	40.6 ± 1.2 ^{a}	44.6 ± 1.0 ^{a}	42.8 ± 2.1 ^{a}	43.5 ± 2.3 ^{a}	44.6 ± 1.4 ^{a}	44.9 ± 1.2 ^{ab}	46.1 ± 1.0 ^{b}	45.8 ± 2.0 ^{a}	43.8 ± 2.0 ^{a}	46.6 ± 1.3 ^{b}
Baking loss (%)	13.1 ± 2.4 ^{c}	7.3 ± 3.0 ^{c}	7.3 ± 0.8 ^{c}	6.4 ± 3.1 ^{c}	11.2 ± 2.1 ^{c}	11.2 ± 1.0 ^{c}	11.3 ± 2.1 ^{c}	8.7 ± 2.0 ^{c}	8.8 ± 1.4 ^{c}	8.9 ± 1.2 ^{c}	7.3 ± 2.8 ^{c}	8.1 ± 2.3 ^{c}	9.4 ± 2.5 ^{c}
Volume (mL)	1193.9 ± 5.56 ^{d}	749.6 ± 43.4 ^{de}	755.4 ± 23.6 ^{de}	931.6 ± 21.1 ^{d}	752.1 ± 27.3 ^{de}	761.5 ± 27.6 ^{de}	945.8 ± 11.3 ^{d}	782.1 ± 27.6 ^{e}	790.3 ± 12.5 ^{e}	956.5 ± 27.3 ^{d}	792.6 ± 11.3 ^{e}	821.2 ± 25.3 ^{e}	1052 ± 12.5 ^{d}
Specific Volume (mL/g)	2.82 ± 1.23 ^{f}	1.1 ± 0.2 ^{f}	1.2 ± 0.2 ^{f}	1.4 ± 0.1 ^{f}	1.2 ± 0.6 ^{f}	1.3 ± 0.0 ^{f}	1.5 ± 0.2 ^{f}	1.4 ± 0.1 ^{f}	1.4 ± 0.1 ^{f}	1.6 ± 0.1 ^{f}	1.5 ± 0.2 ^{f}	1.9 ± 0.0 ^{f}	2.4 ± 0.1 ^{f}

Different letters in subscript show they are significantly different from each other.

Table 1 shows variation in different characteristics of the bread when different amounts of the GH with promoters are used with respect to standard bread. The table shows a mean value of 3 sample observations (n = 3), with ANOVA at 95% confidence interval and an alpha of 0.05. Different letters in subscript show they are significantly different from each other, while the same letters in subscript mean that they are non-significantly different from each other.

Table 2 shows different characteristics of the bread when yeast as a partial replacement is used with respect to the standard bread. The yeast concentration varied from 25% to 75%. The table shows a mean value of 3 sample observations (n = 3), with ANOVA at 95% confidence interval and an alpha of 0.05. Different letters in subscript show they are significantly different from each other, while the same letters in subscript mean that they are non-significantly different from each other.

3.2. Analysis of Pores and Effect on Hardness for GH Bread with Promoters

Table 3 and Figure 3 show the pore size analysis of standard bread versus GH bread with promoters (20–70%). According to the square size, the pores were separated. The smaller ones ranged in size from 0.1 to 2 mm^2, and the medium ones ranged in size from 3 to 6 mm^2, while the larger ones were 10 to 11 mm^2. The GH bread with promoters had pores that were very similar to those of regular yeast bread (Table 3). Compared with typical yeast bread, the 60% to 70% GH had more pores. The number of small pores in the standard yeast bread was 82, while for the GH bread with promoters (20–70%) it ranged from 71 to 89. Additionally, it was discovered that the quantity of big, medium, and tiny pores in the GH bread with promoters (20–70%) was roughly equivalent to that in the yeast bread. Henceforth, it can be verified that when GH with promoters are used, this can produce leavening effects with different pore sizes in the leavened GH bread. Moreover, the pore size analysis of the GH bread displayed in Figure 3 using cut sections reveals that the 60% and 70% GH breads with promoters performed better than the other GH breads with promoters (20% and 40% GH).

Table 3. Pore size analysis of standard bread versus the GH breads with promoters (20–70%).

Bread Type	Pore Class (mm^2)				
	Small (0.10–2.00)	Somewhat Medium (2.01–3.00)	Medium (3.01–6.00)	A Little Bigger (6.00–10.00)	Large (10.00–11.00)
Standard	82.3 ± 0.0 ^{a}	7.8 ± 1.0 ^{b}	7.8 ± 1.0 ^{b}	1.8 ± 0.0 ^{c}	0.4 ± 0.0 ^{c}
20% GH	71.6 ± 2.0 ^{a}	8.8 ± 1.0 ^{b}	11.5 ± 1.2 ^{b}	1.0 ± 0.2 ^{c}	0.8 ± 0.0 ^{c}
40% GH	80.3 ± 2.7 ^{a}	5.1 ± 1.3 ^{b}	4.7 ± 1.5 ^{b}	2.7 ± 0.0 ^{c}	0.7 ± 0.0 ^{c}
60% GH	87.8 ± 2.1 ^{a}	5.2 ± 1.2 ^{b}	5.5 ± 1.7 ^{b}	2.4 ± 0.0 ^{c}	0 ± 0.0 ^{c}
70% GH	89.0 ± 2.7 ^{a}	5.4 ± 1.2 ^{b}	5.6 ± 1.3 ^{b}	2.8 ± 1.1 ^{c}	0.2 ± 0.0 ^{c}

Different letters in subscript show they are significantly different from each other.

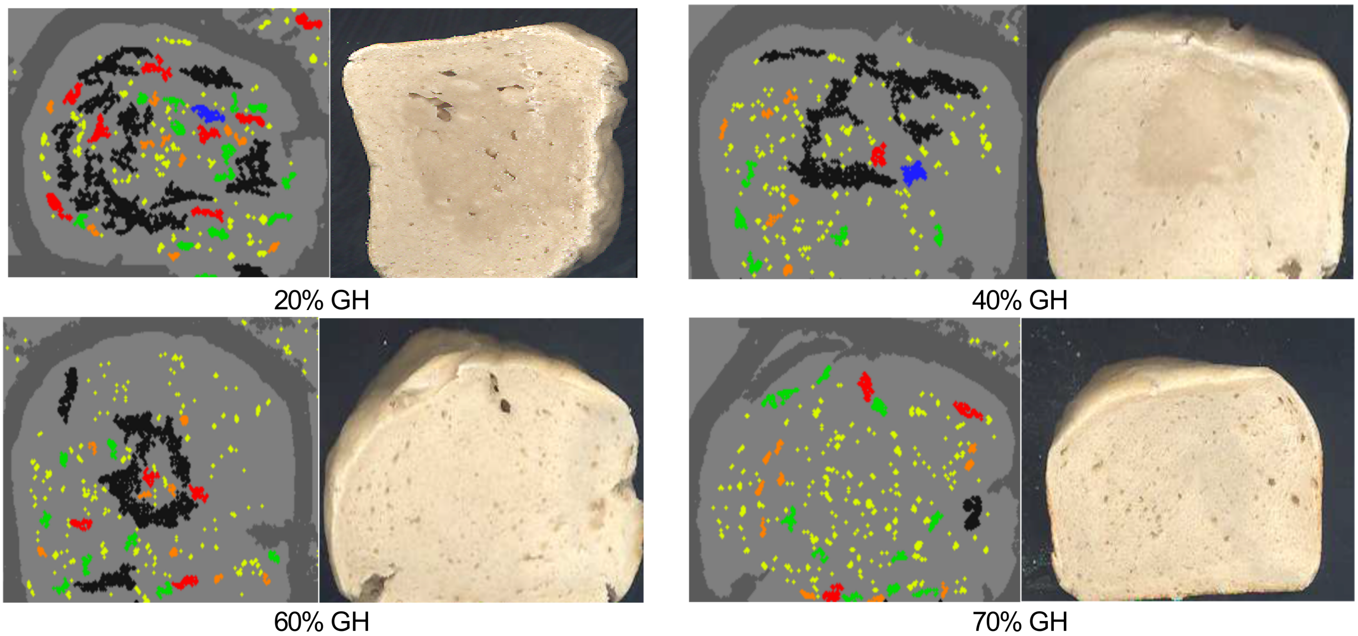

Figure 3. Pore size analysis of GH breads with promoters (20–70%). The figure shows the cross-sectional structure of the bread when breads are cut into thin slices for the GH range from 20% to 70%.

Table 3 shows the different pore sizes of the bread prepared with promoters with respect to the standard bread. The table shows a mean value of 3 sample observations (n = 3), with ANOVA at 95% confidence interval and an alpha of 0.05. Different letters in subscript show they are significantly different from each other, while the same letters in subscript mean that they are non-significantly different from each other.

Table 4 shows the tabular data of the pore size analysis of standard yeast bread versus the GH bread with promoters (20–70%) and 25–75% yeast as a partial replacement. Compared with the standard yeast bread, the combination of GH with promoters (20–70%) and yeast (25–75%) showed a higher number of pores. The number of small pores in the standard yeast bread was 82, while for the GH bread with promoters and yeast the number ranged from 89 to 93. Additionally, it was discovered that in the GH bread with the promoters and yeast combination, the number of tiny and slightly bigger pores was considerably greater in comparison with the conventional yeast bread. The best combinations were obtained with 70% GH + 50% yeast and 70% GH + 75% yeast. Also, it was found that when the percentage of GH along with yeast increased, a significant increase in the pore size was observed, especially in the smaller pores (Table 4). Therefore, the findings imply that during baking, gas hydrates contribute to the leavening of the bread. Figure 4 shows the pore size analysis cut section images of 20% and 40% GH bread with promoters and 25–75% yeast as partial replacement. The cut section pore analysis reveals that 40% GH breads with different amounts of yeast as a partial replacement (25–75%) were better in appearance and in number of pores than the 20% GH and yeast combination (25–75%).

Table 4. Pore size analysis of standard bread versus the GH breads with promoters (20–70%) and 25–75% yeast as partial replacement.

Bread Type	Small (0.10–2.00)	Somewhat Medium (2.01–3.00)	Pore Class (mm^2) Medium (3.01–6.00)	A Little Bigger (6.00–10.00)	Large (10.00–11.00)
Standard	82.3 ± 0.0 [a]	7.8 ± 1.0 [c]	7.8 ± 1.0 [c]	1.8 ± 0.0 [e]	0.4 ± 0.0 [e]
20 + 25% yeast	89.6 ± 1.0 [a]	5.1 ± 1.0 [c]	4.5 ± 1.5 [c]	0.6 ± 0.0 [e]	0.0 ± 0.0 [e]
20 + 50% yeast	89.7 ± 1.0 [a]	7.2 ± 1.4 [c]	4.9 ± 1.2 [c]	4.0 ± 0.7 [e]	0.4 ± 0.0 [e]
20 + 75% yeast	89.8 ± 2.1 [a]	7.8 ± 1.0 [c]	4.9 ± 1.0 [c]	4.1 ± 0.1 [e]	0.4 ± 0.0 [e]
40 + 25% yeast	86.5 ± 2.8 [a]	4.6 ± 1.7 [c]	4.6 ± 1.3 [c]	3.4 ± 1.2 [e]	0.8 ± 0.0 [e]
40 + 50% yeast	88.9 ± 3.0 [a]	6.3 ± 1.2 [c]	7.6 ± 1.2 [c]	2.1 ± 0.0 [e]	0.4 ± 0.0 [e]
40 + 75% yeast	92.2 ± 2.1 [b]	5.4 ± 1.0 [c]	5.4 ± 1.1 [c]	2.8 ± 0.0 [e]	0.4 ± 0.0 [e]
60 + 25% yeast	89.9 ± 2.6 [a]	5.5 ± 1.2 [c]	4.1 ± 1.1 [c]	3.2 ± 1.3 [e]	0.4 ± 0.0 [e]
60 + 50% yeast	90.6 ± 2.1 [b]	5.6 ± 1.0 [c]	4.6 ± 1.4 [c]	3.4 ± 1.0 [e]	0.4 ± 0.0 [e]
60 + 75% yeast	93.4 ± 2.0 [b]	6.2 ± 1.2 [c]	5.0 ± 1.2 [c]	3.9 ± 1.0 [e]	0.4 ± 0.0 [e]
70 + 25% yeast	90.4 ± 2.0 [b]	6.3 ± 1.2 [c]	5.7 ± 1.3 [c]	3.9 ± 1.1 [e]	0.4 ± 0.0 [e]
70 + 50% yeast	90.7 ± 2.7 [b]	6.6 ± 1.4 [c]	5.7 ± 1.2 [c]	3.9 ± 1.0 [e]	0.4 ± 0.0 [e]
70 + 75% yeast	93.5 ± 2.8 [b]	7.3 ± 1.7 [c]	12.7 ± 1.4 [d]	3.9 ± 1.0 [e]	0.3 ± 0.0 [e]

Different letters in subscript show they are significantly different from each other.

Table 4 shows the pore classification of the breads prepared with the combination of yeast and GH in different amounts ranging from 20% to 70%. The table shows a mean value of 3 sample observations (n = 3), with ANOVA at 95% confidence interval and an alpha of 0.05. Different letters in subscript show they are significantly different from each other, while the same letters in subscript mean that they are non-significantly different from each other.

Figure 5 shows the pore size analysis cut section images of 60% and 70% GH with promoters and 25–75% yeast as a partial replacement. The cut section pore analysis reveals that 70% GH and 60% GH with different amounts of yeast as partial replacement (25–75%) were more or less the same in appearance, but the number of small pores was higher in the 70% GH and yeast combination (25–75%). The application of a combination of CO_2 GH with promoters and partial yeast as a leavening agent further clarified that CO_2 GH can produce leavening individually as well. If this were not the case, the results obtained with the combination effect of yeast and CO_2 GH would not indicate a higher number of pores via the pore size analysis of the bread.

Figure 6 shows the hardness profile of standard and GH bread with promoters (20–70%). When the bread slices were tested for hardness using texture profile analysis, the hardness of the bread made with GH was found to be substantially greater than that of the conventional yeast bread. The standard yeast bread had a hardness of roughly 10.5 N, whereas GH bread with promoters had a higher value of hardness in the range of 25.3 N to 32.9 N. However, among all the GH breads with promoters, the 70% GH bread exhibited the lowest hardness (25 N), followed by the 60% and 40% GH breads with promoters. Figure 7 shows the hardness profile of standard and GH bread (20–70%) with promoters and 25–75% yeast as a partial replacement. The combination of yeast and GH with promoters significantly affected the hardness values of the bread. The GH bread with promoters (20–70%) + yeast bread (25–75%) gave significantly lower (p = 0.23) values of hardness. The standard yeast bread had a hardness of 10 N, whereas GH bread with promoters and yeast as partial replacement had a value of hardness in the range of 10 N to

30 N. However, among all the GH breads with promoters and yeast as a partial replacement, the 70% GH + 75% yeast bread exhibited the lowest hardness (10 N), which was comparable to the standard bread, followed by the 70% GH + 50% yeast bread (13 N) and 70% GH + 25% yeast bread (15 N) with promoters.

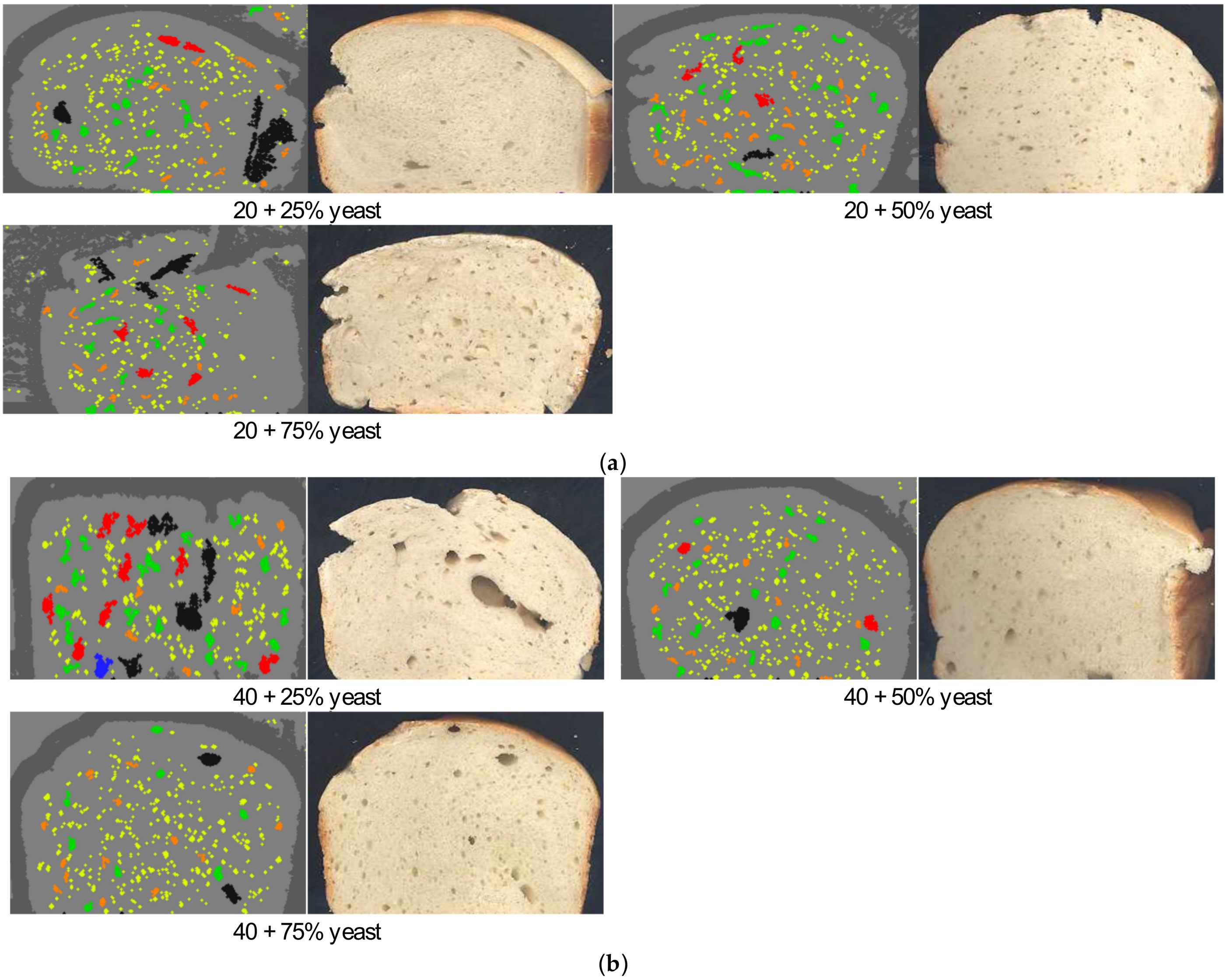

Figure 4. Pore size analysis of (**a**) 20% and (**b**) 40% GH breads with promoters and 25–75% yeast as partial replacement. The figure shows cross-sectional images of the bread pores when they are prepared with different amounts of yeast and GH.

The figure shows variations in the hardness of the bread when the GH percentage was varied.

The figure shows that when yeast is added along with the GH in different percentages, the hardness of the bread was consecutively decreased.

Due to a lack of fermentation in the GH bread with promoters, the conversion of starch into simple sugars was hampered, which also had an impact on the GH bread's moisture content. It is possible that the GH bread's thick crust caused the crumb to retain moisture, giving it a firmer texture [33,35,40]. The gluten network may have also contributed to the tougher GH bread with promoters. In one way or another, yeast's chemical reactions and biological activity influence the gluten's structure, which in turn impacts the gluten's ability to retain gas. Thus, by releasing moisture during baking, yeast and gluten may contribute to the development of softer bread [33,34,40,41]. Therefore, in order to make GH bread with promoters that has a much softer texture, i.e., when enhancing the GH bread recipe, the bread's hardness is a crucial component that should be considered. It was challenging to tie the present work to ongoing research because such study is quite limited. However, one of our recent research projects has been undertaken in the field of baking [8,9,30], and this research is an extension of our previously published work [32]. The results showed that there was still potential for improvement in the GH bread recipe with promoters [32]. It is feasible to create gas hydrates on-site by putting a reactor in the bakery. Furthermore, the sole ingredients used in the creation of GH are water and CO_2. There are no additional chemicals necessary for manufacture. By using GH, proofing time may be eliminated, saving money on the expense of the proofing chamber necessary for yeast fermentation. Also, with GH, no such dough storage is necessary, and the product may be cooked directly. However, the manufacture of GH necessitates high pressure and low temperature, which might increase the energy costs. However, with the use of CO_2 GH as a leavening agent, the time of the baking process becomes more variable, and this method may be employed successfully in small branch bakeries.

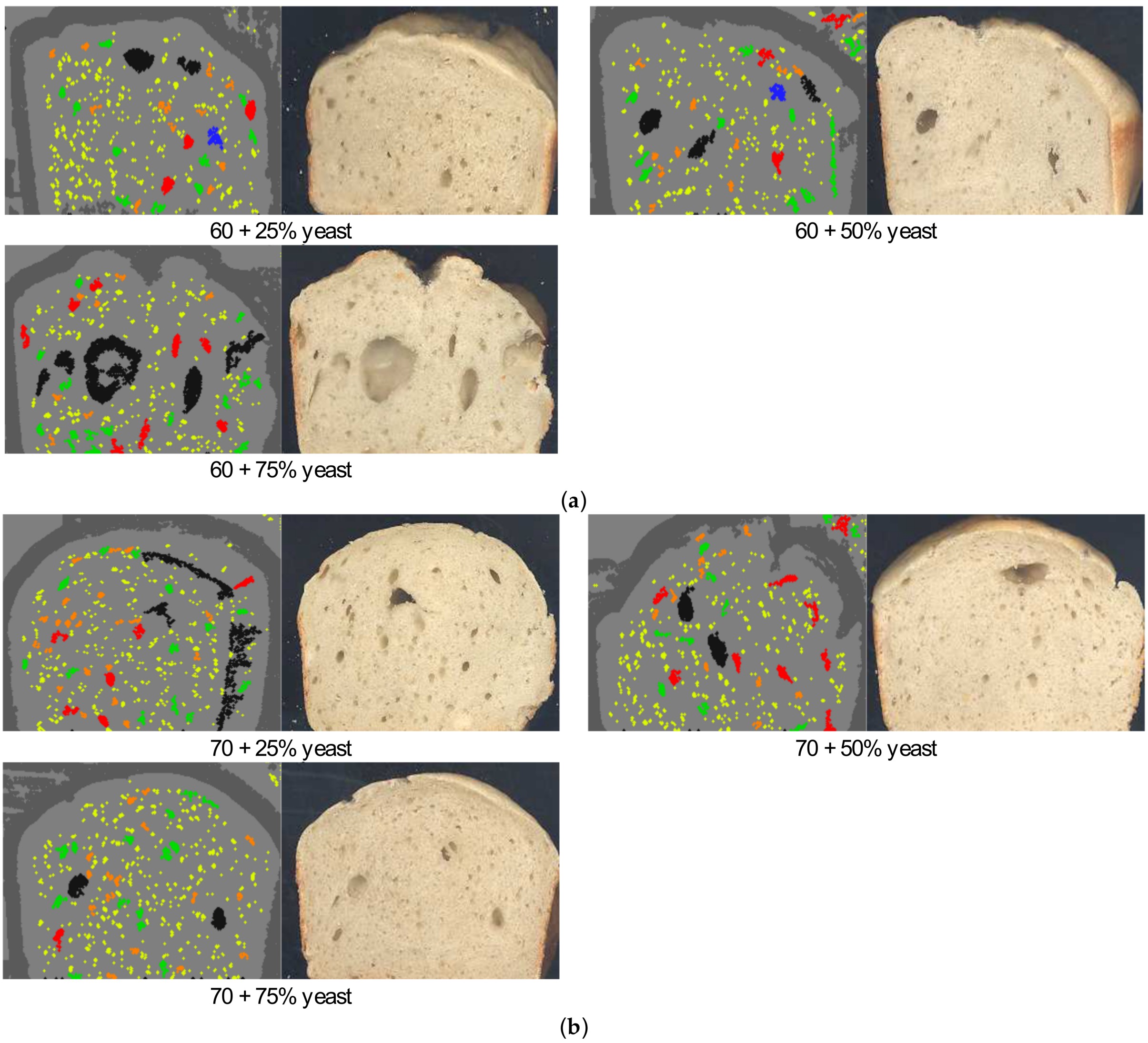

Figure 5. Pore size analysis of (**a**) 60% and (**b**) 70% GH breads with promoters and 25–75% yeast as partial replacement. The figure shows that there was improvement in the bread pores when the cross-sectional image was taken.

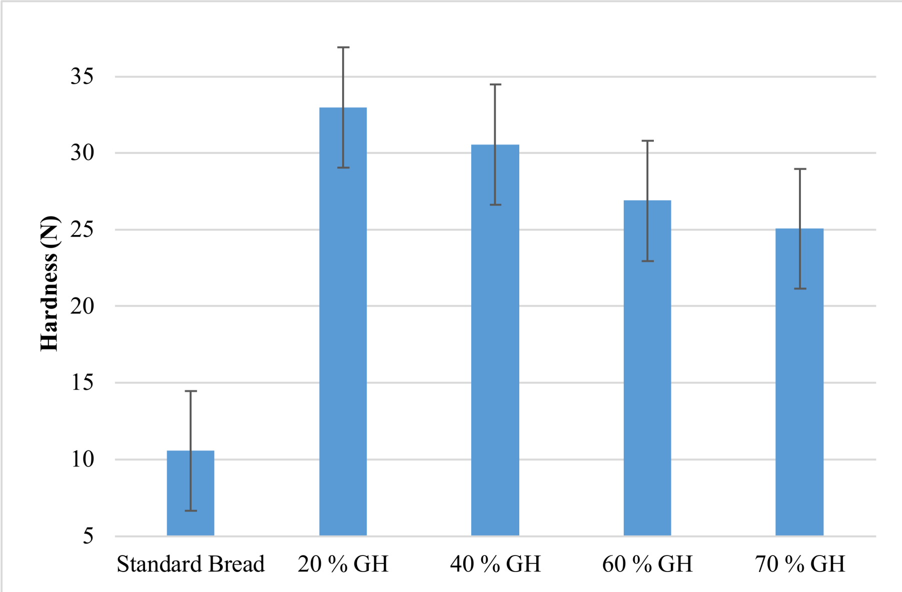

Figure 6. Hardness profile of standard and GH breads with promoters (20–70%).

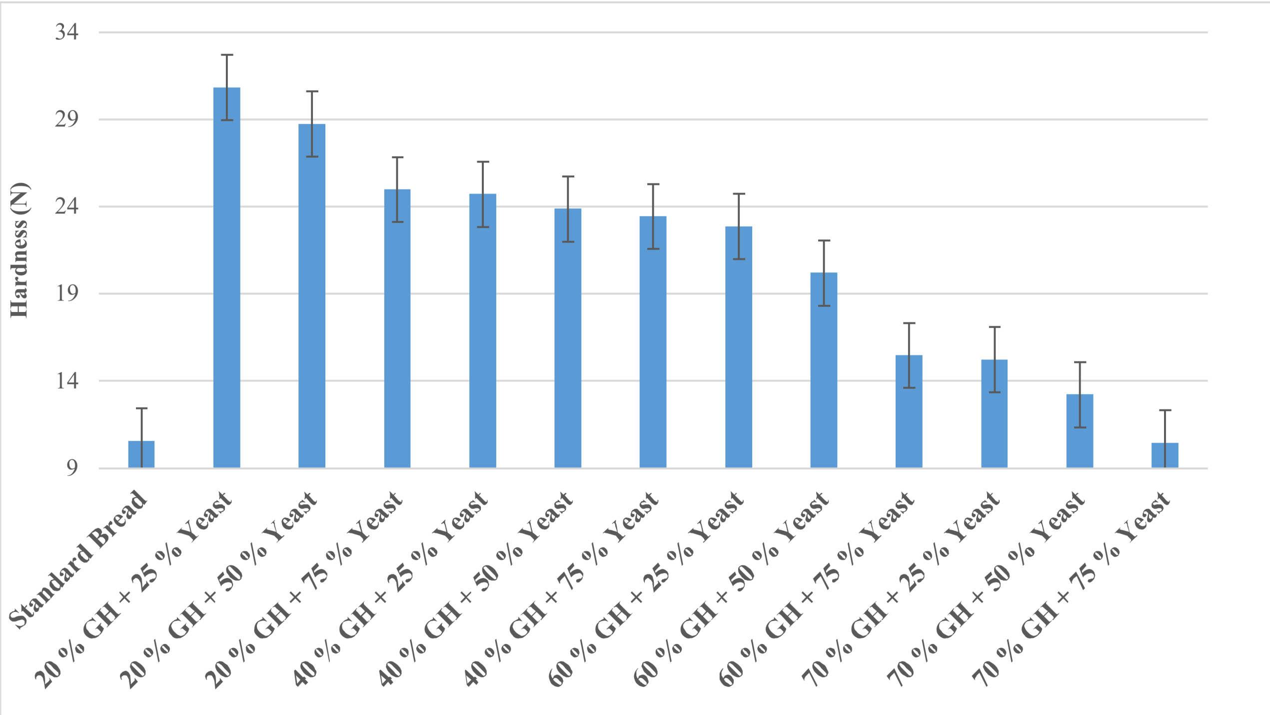

Figure 7. Hardness profile of standard and GH breads (20–70%) with promoters and 25–75% yeast as partial replacement.

4. Conclusions

One of the challenges that this research attempts to solve is the baking industry's use of CO_2 GH as a leavening agent for wheat bread. Among the main benefits of CO_2 GH as a leavening agent would be that they are a desirable alternative for the continuous manufacture of yeast-free leavened dough, are chemical-free, and have no impact on the sensory qualities of the baked goods.

The breads with 60% and 70% GH with promoters had the best outcomes in terms of volume and pore size when compared with standard yeast bread. The GH bread with promoters had baking losses that were equivalent to the quality of traditional yeast bread. Moreover, when a combination of CO_2 GH with promoters (20–70%) and yeast (25–75%) was used in the study, the results obtained with respect to pore size and hardness were found to be more effective. The best combinations were obtained with 70% GH + 50% yeast and 70% GH + 75% yeast. Also, it was found that when the percentage of GH along with yeast increased, a significant increase in the pore size was observed, especially in the smaller pores. Therefore, the findings imply that during baking, gas hydrates contribute to the leavening of the bread. Hence, based on the results, the 60% and 70% GH breads with promoters have better chances of improvement with additional alterations to the manufacturing process. Including other ingredients that promote the production of gluten such as pentosans in the bread in addition to CO_2 GH might help to improve the GH bread recipe further. Additionally, it was found that certain changes need to be made to the GH reactor's architecture and the process for manufacturing GH bread with promoters during the kneading stage in order to take into account the results obtained.

Author Contributions: Conceptualization, S.S. and B.H.; methodology, S.S., A.M.K. and V.Z.; software, S.S.; validation, S.S., V.Z. and B.H.; formal analysis, S.S. and A.M.K.; investigation, S.S., A.M.K., T.C. and M.M.; resources, B.H., A.D., T.C., M.M. and B.G.; data curation, S.S.; writing—original draft preparation, S.S. and B.H.; writing—review and editing, S.S., V.Z. and B.H.; visualization, S.S., V.Z., B.G. and M.J.; supervision, B.H. and M.J.; project administration, B.H.; funding acquisition, B.H. and A.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by AiF 21084 N within the program for promoting the Industrial Collective Research (IGF) of the German Ministry of Economics and Energy (BMWi).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The related data are included in the manuscript.

Acknowledgments: This IGF project of the FEI is/was supported via AiF 21084 N within the program for promoting the Industrial Collective Research (IGF) of the German Ministry of Economics and Energy (BMWi).

Conflicts of Interest: The authors of the manuscript declare that there are no conflict of interest.

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