

STUDY OF BY-PRODUCTS FROM THE BAKING INDUSTRY

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Abstract. *Food waste in the baking industry presents a significant sustainability challenge. This study investigates the valorization of stale bread through enzymatic hydrolysis to produce glucose syrup and assesses its feasibility as a brewing adjunct. A two-day hydrolysis protocol using alpha-amylase and glucoamylase was evaluated at different bread:water (mass:mass) ratios. Results showed the 40:200 ratio yielded the highest product concentration (18.7% reducing sugars; 19.8 °Brix), whereas the 20:220 ratio demonstrated the most efficient conversion yield per gram of bread. Subsequently, the produced bread-based syrup was used for a 30% malt substitution in a Light Ale, compared against a 100% malt control and a 30% stale bread flour. The bread-based syrup beer exhibited the highest pre-fermentation reducing sugar content (5.19%) and superior foam stability. All formulations achieved comparable final Alcohol by Volume levels (3.2–3.4%). This study concludes that enzymatic hydrolysis is a successful valorization strategy for stale bread, producing a viable syrup for 30% malt substitution in brewing.*

Keywords. *Bakery Industry, Food Waste, Circular Economy, Glucose Syrup, Sustainable Practices, Innovation, High-Value-Added Products.*

Introduction

The Brazilian baking sector is a cornerstone of the national economy. A notable expansion was recorded between 2023 and 2024, with the sector experiencing 10.92% growth and reaching a revenue of R\$153.36 billion. This performance, representing a surge of R\$14.82 billion (ABIP, 2025), reflects an ever-increasing demand for its products. This growth is particularly significant because the sector, comprising over 70,000 establishments, is not only economically significant but also a key source of employment.

Despite being one of the world's largest food producers, Brazil faces a paradox that undermines its economy. While the nation achieves record agricultural output (IBGE, 2025), it still struggles with critical levels of food waste (UN, 2024). A considerable amount of unsold bread loaves is discarded, which represents not only a loss of valuable resources, such as labor, ingredients, water, and energy, but also contributes significantly to greenhouse gas emissions (FAO, 2020).

This challenge highlights the need for innovative solutions to repurpose bread waste into high-value-added products. In 2024, food industry revenue reached R\$ 1.277 trillion, a 9.98% nominal increase from the prior year, representing 10.8% of the nation's GDP (ABIA, 2025). Repurposing discarded bread into new products could significantly

mitigate waste and generate economic value, aligning with both sustainability goals and industry innovation.

The utilization of discarded bread that is not microbiologically compromised can be realized via enzymatic hydrolysis of starch. This process is carried out in two stages. In the first stage, liquefaction occurs with the use of alpha-amylase to cleave the alpha-1,4 bonds of complex starch polysaccharides, yielding shorter dextrin chains. In the second stage, saccharification occurs with the use of the glucoamylase enzyme to hydrolyze the dextrin into glucose (Riaukaite *et al.*, 2019). The resulting hydrolysate can then be employed as a brewing adjunct, such as wheat or barley (Dall'Acua *et al.*, 2025).

Parameters such as the bread-to-water ratio, initial pH, enzyme concentration, and stirring frequency play a crucial role in optimizing starch hydrolysis and conversion. Throughout the process, pH and temperature adjustments are necessary at different stages to maintain optimal enzymatic activity (Riaukaite *et al.*, 2019). Additionally, reaction time and enzyme dosage must be carefully controlled to maximize yield and ensure product quality.

To extract the most fermentable sugar in beer production, the mashing process must be carefully controlled. The key is to balance time and temperature to allow starch gelatinization (Coelho *et al.*, 2024). Once the gelatinized starch is accessible, enzymes can efficiently convert it into fermentable sugars (Langenaeken *et al.*, 2020). Using pre-hydrolyzed bread would increase sugar extraction efficiency. Since the extraction is already complete, the hydrolyzed syrup can be added directly to the boil, much like brewers add sugar, honey, or other fermentable adjuncts.

Brazilian legislation (Normative Instruction no. 65/2019, MAPA (2019)) provides the classification of beer and the utilization of brewing adjuncts, ingredients used as substitutes for malted barley. The brewing adjuncts are legally defined to include "honey and ingredients of vegetable origin, sources of starch and sugars, suitable for human consumption as food." Consequently, glucose syrup, which is commercially derived from vegetable starches such as corn, falls within this classification, permitting its use as a fermentable sugar source in brewing.

A general classification for "cerveja" (beer) stipulates that the beverage must be produced from a wort containing a minimum of 55% malted barley and a maximum of 45% total brewing adjuncts, calculated by weight relative to the original extract. This 45% threshold represents the absolute maximum cumulative limit for all non-malted barley ingredients combined (unmalted cereals, syrups, honey). While the total amount of brewing adjuncts can be up to 45%, there is a specific sub-limit: adjuncts from non-barley/non-wheat sources (like syrups or corn) cannot exceed 25% of the total.

Objectives

This work aims to investigate enzymatic processes utilizing stale bread as a sustainable feedstock for the development of higher-value products. Specifically, the research focuses on determining the operational parameters that influence the enzymatic hydrolysis of starch into glucose syrup, while concurrently exploring the feasibility of producing beer from waste bread as an alternative valorization strategy.

Development

The bread loaves used in this project were prepared using the sponge and dough method. The ingredients were purchased at the local market and are described in Table 1. The sponge was prepared 16 h in advance. The final dough was mixed for 4 min at the 1st speed for hydration and 9 min at the 2nd speed for dough development in a mixer (Suprema-R.15, Brazil). At the end of the mixing, the dough temperature was 23 °C. After resting for 10 min, the dough was divided into 10 portions of 320g. The portions were rounded and shaped manually.

Proofing was conducted in an ultra-freezer (model Uki-05, Klimaquip, Brazil) for 60 min at 36 ± 1 °C, and baking was performed in a deck oven (model ECOPOWER, Prática, Brazil) for 20 min at 250°C on the base (deck) and 230 °C on the top (ceiling). The breads were cooled at room temperature for 2 h and stored at 25° C in a BOD (model SSB.O.Du-350L, SolidSteel, Brazil) for 4 days until they hardened, and then frozen in domestic equipment (model DA550, Metalfrio, Brazil) at -20°C.

Table 1: Bread formulation.

Ingredients	Sponge (30%)		Final	
	%	g	%	g
White flour	50	300	100	1400
Whole wheat flour	50	300	-	-
Water	60	360	60	840
Dried yeast	0,1	0,6	1,7	23,4
Salt	0,2	1,2	2,8	38,8
Sponge	-	-	68,7	961,8
Total		961,8		3264,4

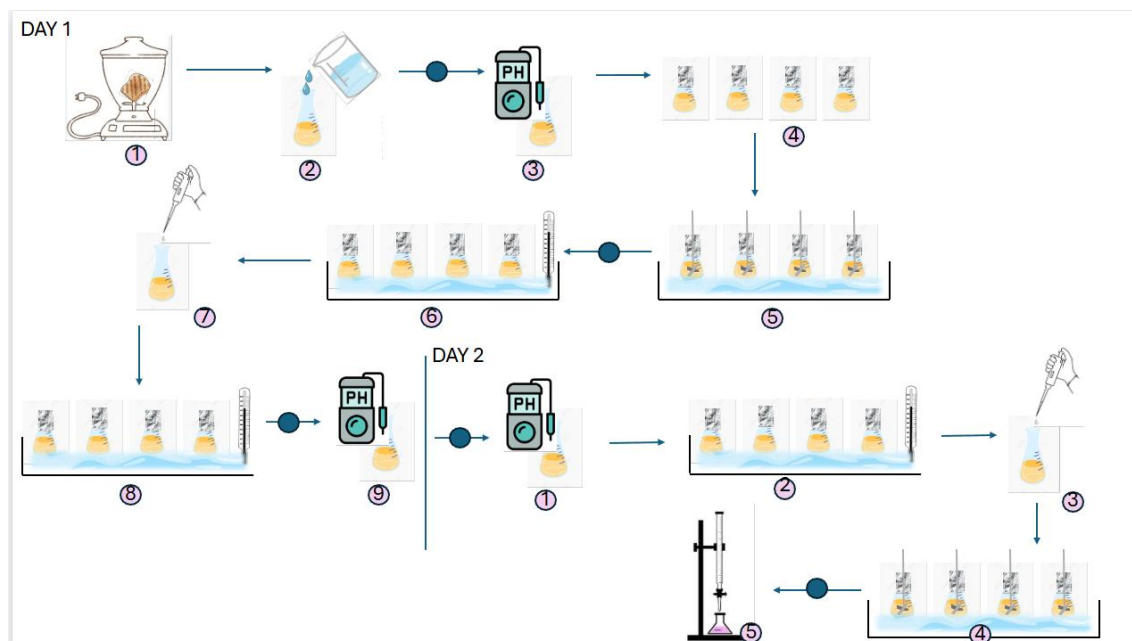
*Percentages expressed on a 100% total flour basis.

The proximate composition of the bread sample was analyzed after 4 days of storage. The chemical and physicochemical properties of the samples were determined in triplicate. Moisture content was determined gravimetrically in a forced-air oven (model 420-4D, Ethik, Brazil) at 105°C until constant weight. Protein content was quantified by the Kjeldahl method; reducing sugars were determined by the Fehling method; and ash content was assessed by incineration in a muffle furnace (model Q.318.24, Quimis, Brazil) at 550°C (IAL, 2008). Total dietary fiber was analyzed using the enzymatic-gravimetric method (Prosky *et al.*, 1992) with a Megazyme kit (K-TDFR-100A), with thermostable alpha-amylase, protease, and amyloglucosidase. The pH and water activity were measured using a pH meter (model PG200, GEHAKA, Brazil) and a water activity meter (model 4TE, AquaLab, Brazil), respectively.

Before the enzymatic hydrolysis process, stale bread was thawed and milled in a coffee grinder (Hamilton Beach, Brasil) for 20 s. The particle size distribution was determined by a set of sieves (16, 25, 35, 40, 60, 80, 115, 150 mesh), which correspond to sieve openings of 1.18 mm, 0.71 mm, 0.5 mm, 0.425 mm, 0.25 mm, 0.177 mm, 0.125 mm, and 0.106 mm, respectively.

The hydrolysis process was carried out with the enzymes alpha-amylase Starzyme AA 240 and glucoamylase MaxiFerm GA, kindly donated by Prozyn BioSolutions for Life. A preliminary test was conducted with a ratio of 40 g of stale bread and 200 g of water. It was fundamental for establishing the process route described in Figure 1, a two-day protocol for converting starch into glucose syrup. The procedure was performed in duplicate to ensure accuracy and reproducibility.

Figure 1: Stages of enzymatic hydrolysis of stale bread.



Enzymatic hydrolysis of stale bread, in which on Day 1: (1) Bread grinding; (2) Bread hydration; (3) pH adjustment = 6.0; (4-5) Bath at 45°C and agitation of 2.5 Hz for 20 min; (6-7) Bath at 65°C and addition of alpha-amylase; (8) Bath at 90°C for 1 h; (9) pH adjustment = 4.3. Day 2: (1) pH reading; (2) Bath at 50°C; (3) Addition of glucoamylase; (4) Bath at 65°C and agitation of 2.5 Hz for 10h.

The process began by grinding stale bread into a fine, homogeneous flour using a coffee grinder. The bread flour sample (20 g, 30 g, and 40 g) was hydrated with distilled water to a total mass of 240 g. The pH of the mixture was adjusted to 5.6–6.2 with HCl solution (0.861 mol/L) to ensure optimal enzyme activity. The mixture was agitated at a constant 2.5 Hz and placed in a 45°C water bath for 20 min. The temperature was then raised to 65°C, and 1 mL of an alpha-amylase solution (1.20 g enzyme in 100 mL of distilled water) was added per 20 g of bread. Subsequently, the temperature was increased to 90°C and maintained for one hour to promote starch gelatinization and liquefaction, concluding the Day 1 procedure.

On Day 2, the temperature was adjusted to 50°C, pH was set to 4.3 with HCl solution (0.861 mol/L), and 1 mL of a glucoamylase solution (0.62 g enzyme in 100 mL of distilled water) was added per 20 g of bread. The mixture was incubated in a water bath at 65°C for 10 to 12 h, with continuous agitation at 2.5 Hz. This prolonged period was intended to ensure the complete conversion of dextrin into glucose. A final soluble solid was measured.

The mixture of bread and water was analyzed for soluble solids content (°Brix) during enzymatic hydrolysis process after the hydration (2), initial heating (4), and liquefaction (8) stages on Day 1; and at the beginning of the process (1) and the end of

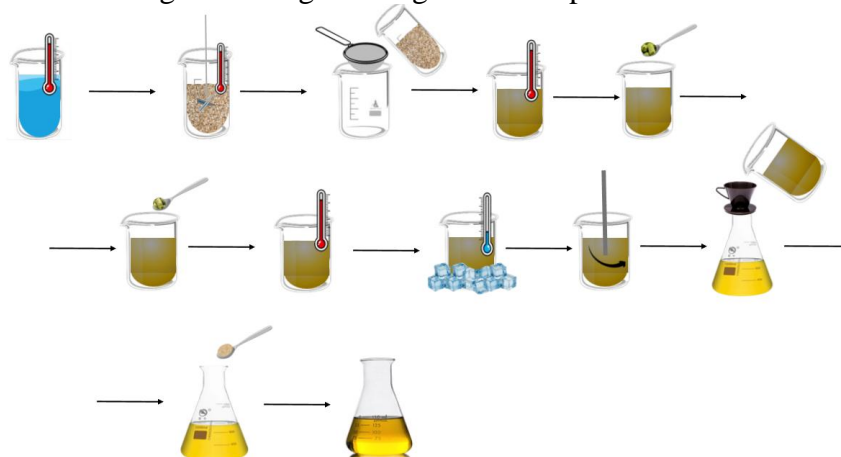
the process (4) on Day 2. To prevent moisture loss and maintain consistent conditions, the flasks were covered with aluminium foil throughout the entire process.

The hydrolysis product was subjected to the Fehling test (IAL, 2008) to determine the reducing sugar content. Moisture content was determined in a forced-air oven at 105 °C for 3 h (model Q314M253, Quimis, Brazil), in triplicate.

The best proportion of stale bread and water for the enzymatic hydrolysis process was used to obtain a large amount of bread-based syrup to produce beer. This syrup was evaluated as a malt substitute in brewing, as its sugar content, moisture, and soluble solids were in conformity with regulatory standards. This composition enabled 30% substitution of the traditional malt requirement in the beer production process (MAPA, 2019).

Beers produced using either bread-based syrup or stale bread flour as a malt adjunct were compared with control beer (Table 2). The control was produced according to the Light Ale formulation from Piquiri Brewshop (2025), which was modified to obtain 2 L of beer (Figure 2). The Light Ale style was selected for its lighter-bodied profile, simplified formulation, and suitability for laboratory-scale production.

Figure 2: Stages of Ligh Ale beer production.



Beer production procedure, in which: (1) Heat water to 66.4°C; (2) Add milled malt. Hold at 62°C for 60 min (measure °Brix), raise the temperature to 75.6°C and hold for 10 min (measure °Brix); (3) Sparge the grain with 1.42 L of water at 75.6°C, Rest for 30min, then perform a 2nd recirculation; (4) Bring to a boil; (5) After 15 min, add hops (bitterness); (6) After 45 min, add hops (aroma); (7) Boil for 5 min; (8) Cool to 60°C (measure °Brix); (9) Create a counter-clockwise vortex (whirlpool) to settle proteins; (10) Rest for 20 min; (11) Filter; (12) Ferment for one week at 20°C; (13) Mature for one week at 0 to 5°C.

Table 2: Light Ale (LA), Bread-based syrup (BBS) and Stale bread flour (SBF) beer formulations.

	LA	BBS	SBF
Ingredients	(g)	(g)	(g)
Pilsen malt	370.0	259.0	259.0
Stale bread flour		111.0	
Bread-based syrup			393.1
Saaz hops	3.0	3.0	3.0
Magnum hops	2.0	2.0	2.0
Water	1264.0	1264.0	981.9
Yeast	1.15	1.15	1.15
Cristal sugar + water (1:1)	60.0	60.0	60.0
TOTAL	3120.95	3120.95	3120.95

The beer samples were evaluated for physicochemical parameters. Density was measured pre and post-fermentation with a hydrometer. Reducing sugars and soluble solids were determined according to IAL (2008), while alcohol content was determined according to Triboli (1989).

Turbidity was assessed using the ASBC Beer-10 spectrophotometric method (ASBC, 2015), which involved measuring absorbance at 430 nm and 700 nm (model DR 3900, Hach, USA). Samples were classified as clear if $\text{Absorbance}_{700\text{nm}} \leq 0.039 \times \text{Absorbance}_{430\text{nm}}$; otherwise, they were classified as turbid.

The alcohol content was also calculated using the ABV (Alcohol by Volume) equation (Equation 1), based on the difference between density before and after fermentation.

$$\text{ABV (\%)} = (\text{OG} - \text{FG}) \times 131.25 \quad \text{Eq. 1}$$

In which OG is original gravity; and FG is final gravity.

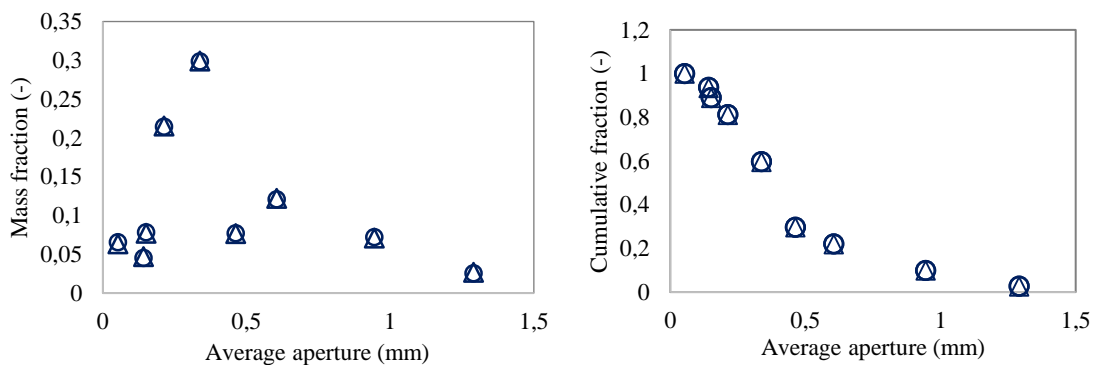
Results and Discussion

This section presents the results obtained during the project.

The composition of the bread was $15.25 \pm 0.26\%$ moisture, $12.05 \pm 0.04\%$ protein, $2.51 \pm 0.04\%$ ash, and 70.18% carbohydrates by difference. This composition included $1.94 \pm 0.01\%$ reducing sugars and $4.00 \pm 0.48\%$ total dietary fiber. The pH was 5.77 ± 0.02 , water activity (A_w) was 0.672 ± 0.004 , and soluble solids was 1.0°Brix (measured in a 1:10 g sample/g water).

The results of the particle size distribution analysis for milled bread are presented in Figure 3. The analysis determined a volumetric mean diameter of 0.126 ± 0.001 mm and a Sauter mean diameter of 0.226 ± 0.001 mm.

Figure 3: Particle size distribution of stale bread flour.



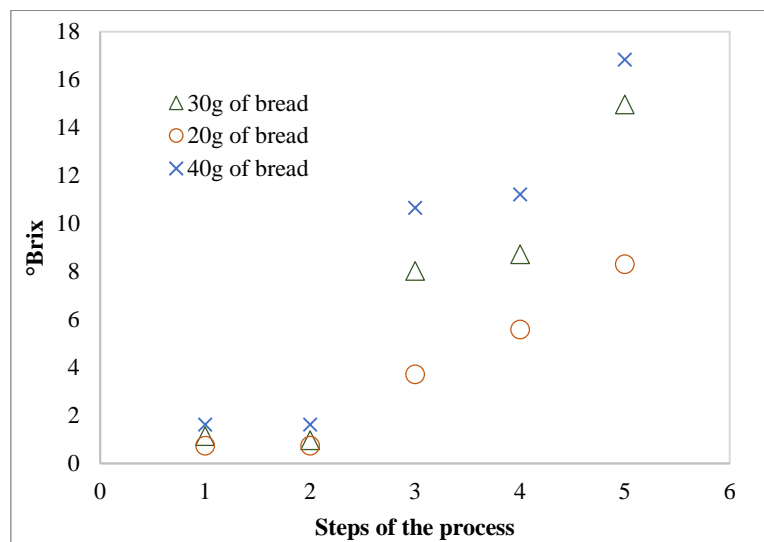
The results of the enzymatic hydrolysis showed that the soluble solids content increased at each stage of the process (Figure 4). Table 3 shows the moisture and reducing sugar content for each bread:water ratio condition and the hydrolysis products can be observed in Figure 5.

Table 3: Reducing sugar (RS), moisture, and soluble solids (SS) content in the hydrolyzed syrups performed with 20:220; 30:210; 40:200 (g bread: g water).

Ratio	RS (%)	Moisture (%)	SS (°Brix)
40:200	$18,7 \pm 0,4^A$	76 ± 1	$19,8 \pm 0,3^A$
30:210	$13,0 \pm 0,3^B$	79 ± 2	$17,5 \pm 1,3^B$
20:220	$10,4 \pm 0,3^C$	$89,9 \pm 0,2$	$9,1 \pm 1,3^C$
Tukey HSD	0,2	2,7	2,4

* Averages in the same column followed by the same letter are not significantly different ($p > 0.05$); Tukey HSD: Tukey's Honestly Significant Difference.

Figure 4: Soluble solids (°Brix) monitored throughout the process.



Soluble solids (°Brix) monitored throughout the process: (1) Grounded bread and water mixture; (2) After 45°C for 20 min; (3) After added alpha-amylase, 90°C; (4) Day 2, after resting; (5) After 65°C and 10 h.

Figure 5: Hydrolyzed syrups performed with 30:210 and 40:200 (g bread: g water).



The hydrolysis assays successfully produced high concentrations of reducing sugars compared to the initial value of 2,29 g/ 100 g bread (dry basis). The obtained values appear to exceed the theoretical maximum yield, suggesting a conversion greater than that possible if all starch were hydrolyzed to glucose. However, it must be considered that water loss during the process may cause the concentration of syrups. The mass losses during the process were $(29.6 \pm 4.5) \%$ based on the initial and final total weights.

The 20:220 ratio, which is the most diluted, shows a sum of moisture and soluble solids approaching 100%. Conversely, the more concentrated ratios result in a larger deviation from 100%. This gap can be attributed to the insoluble solids fraction, primarily protein and fiber, which remain suspended in the syrup.

The higher the amount of bread sample, the higher the sugar level, as shown in the 40:200 condition, which produced the highest sugar concentration (g/L) and resulted in the most potent syrup. Therefore, this syrup was selected for initial feasibility trials as a brewing malt substitute.

In contrast, the 20:220 condition demonstrated the highest conversion yield, proving to be the most efficient process relative to the initial mass of aged bread. Consequently, while the 40:200 syrup was advanced for preliminary viability testing, the superior process efficiency of the 20:220 condition warrants its investigation in future studies, pending positive initial results.

The syrup produced for beer production had $84.05 \pm 0.21\%$ moisture, $13.67 \pm 0.46\%$ reducing sugars, and $13.8 \pm 0.1\%$ soluble solids. The observed process inefficiency can be attributed to two main factors. First, prolonged freezing may have accelerated starch retrogradation, thereby increasing the resistant starch content and consequently impeding enzymatic conversion. Second, the enzymes may have experienced a loss of catalytic activity over time, which would also reduce the overall conversion yield.

The results of the analyses performed on the beers are presented in Table 4. The soluble solids content before fermentation was 10.0°Brix for Light Ale (LA), 9.3°Brix for bread-based syrup (BBS), and 8.2°Brix for stale bread flour (SBF). This trend was not in accordance with the reducing sugar content. The BBS condition presented the highest reducing sugar concentration, which was the most favorable result among the studied conditions. Regardless, further investigation of these results is warranted.

The alcohol content was obtained by alcohol by volume (ABV) and in concentration (g/L). The results are equivalent across different units. Furthermore, the alcohol content, as determined by the methodologies employed, did not differ significantly among the treatments, regardless of their initial sugar concentrations.

Table 4: Reducing sugar (RS) before fermentation, alcohol, and soluble solids (SS) content and density before (D1) and after (D2) fermentation in Light Ale (LA), bread-based syrup (BBS), and stale bread flour (SBF) beer.

Beer	RS (%)	Alcohol (g/L)	SS ($^\circ\text{Brix}$)	D1 (g/mL)	D2 (g/mL)	ABV (% v/v)
LA	4.20 ± 0.06^C	32.1 ± 1.3	6.3 ± 0^B	1.036 ± 0	1.010 ± 0	3.4
BBS	5.19 ± 0.04^A	32.3 ± 1.1	6.25 ± 0.07^B	1.034 ± 0	1.010 ± 0	3.2
SBF	4.61 ± 0.06^B	30.5 ± 1.1	6.8 ± 0^A	1.037 ± 0	1.012 ± 0	3.3
Tukey HSD	0.41	-	0.5	-	-	-

* Averages in the same column followed by the same letter are not significantly different ($p > 0.05$); Tukey HSD: Tukey's Honestly Significant Difference.

The turbidity results for Light Ale, bread-based syrup, and stale bread flour beer classified as turbid all the analyzed samples. According to this analysis, visually (Figure 6), the three samples are similar, exhibiting a comparable yellow/orange color and an observable turbidity. The primary visual distinction lies in the foam characteristics. The hydrolyzed bread sample produced the most significant foam head, which also

appeared more full-bodied and stable than the foam on the standard beer. Conversely, the stale bread flour sample produced very little foam.

Figure 6: Light Ale (middle), bread-based syrup (front), and stale bread flour (background) beer.



Conclusion

Enzymatic hydrolysis of aged bread, using the enzymes alpha-amylase and glucoamylase, was a successful method for producing high concentrations of reducing sugars in stale bread-based syrup. It was observed the initial bread mass and the final sugar production of 40:200 (g bread: g water) resulted in the highest final sugar concentration (g/L). However, the 20:220 condition exhibited the highest conversion yield, producing the higher sugar content per gram of initial stale bread.

The analysis of sugar content, moisture, and soluble solids indicated that it is viable for use as a malt substitute in beer production. The syrup's composition allowed for, in conformity with regulatory requirement, a 30% substitution of the malt in the beer production process.

The objective of investigating the conversion of bread into higher-value products was achieved. The study not only demonstrated the viability of enzymatic hydrolysis for syrup production but also successfully explored its application as an alternative valorisation strategy in beer production. The produced syrup proved to be viable for a 30% malt substitution, fulfilling the goal of developing a sustainable solution for food waste management.

The economic viability of the process should be assessed, focusing on the energy cost and the capital investment required for equipment, particularly tanks and industrial agitators, necessary for large-scale production of the syrup.

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