

Article

Brewing Mainly from Stale Bread: A Pale Ale Case Study

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Abstract: Contemporary environmental concerns have led to the prioritization of sustainable production and material circularity, no matter what the industrial field of activity. Bread waste is a major element of overall food waste since, worldwide, bread remains a widespread staple food. A considerable proportion of bread consumption is of fresh, baked bread, consumed daily, generating substantial amounts of stale bread. Therefore, efforts to reintroduce this waste into the food value chain can make a significant contribution to reaching zero food waste, which is a major target in European countries. Possible ways to produce new raw materials through starch enzymatic hydrolysis include brewing, which is an activity in which incorporating stale bread is of great interest. Mashing parameters in brewing processing are the main focus of this study, primarily the time and temperature required to acquire optimal enzymatic activity for starch-efficient hydrolysis. Extending the mashing time to 290 min, within a temperature range of 45–75 °C, allowed us to replace 50% of the required malt with stale bread, thus obtaining a successful pale ale beer. The incorporation of stale bread in a 50:50 ratio did not affect the overall character of the beer, although the alcohol levels stood around 2% below a standard beer's average level. Depending on the brewer's final goal, this lighter kind of beer may be well-aligned with new consumer trends supporting more sustainable and lower-alcohol beverages.

Keywords: beer; bread waste; circular economy; enzymatic activity; sustainability



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1. Introduction

The food industry has a large share of the responsibility for greenhouse gas emissions and the overall carbon footprint, and is therefore a main target of environmental policies [1]. In March 2020, the European Parliament redesigned its Circular Economy Action Plan, aiming for strategies to reduce waste and develop ideas and techniques to design a new life cycle for inevitable waste products. The Circular Economy Action Plan strives for sustainable products, consumer awareness, circularity in components, reduced waste, and economic vitality for regions and people [2].

Bread represents a basic component of the human diet, with an estimated average consumption of 70 kg/year/capita worldwide [3]. Bulgaria is the European country with the highest consumption per capita (95 kg per year). Other European countries have shown slight decreases in bread consumption, reporting an overall 59 kg/year/capita [4]. Such a demand results in an overproduction of fresh bread for daily consumption and a considerable amount of waste, in the form of stale bread. Bread waste may be justified by bread's relatively short shelf life [5]. The main source of bread waste is found in households, but food retail also represents a considerable source of waste, mainly in restaurants, schools, and bakeries [6]. This waste leads to a critical scenario in the majority of European countries, resulting in a 10% loss/waste of 100 million tonnes baked annually across Europe. For example, the United Kingdom alone estimates that 44% of bread is wasted, meaning 20 million slices per day and representing 292,000 tonnes per year associated with 584,000 tonnes of CO₂ emissions. The Netherlands loses over EUR 400M

associated with bread waste alone [7]. Bread waste composition makes it an ideal precursor to bioproducts generated by fermentation processes [8]. Beer and bread remain at the top of the consumer food hierarchy, historically considered staple items in global diets until recent times. With both requiring a mixture of water and harvested grains, it is valid to suggest that recirculating their waste would reduce the associated potential CO₂ emissions and contribute to a conscious strategy to reduce food waste [9].

Within a circular economy, bread waste is often donated and transformed into animal feed. Nevertheless, some waste is reintroduced into bread dough, used for dried toasts or to produce breadcrumbs. These transformations upcycle stale bread back into the food value chain. But a considerable amount of this nutritious waste is left for anaerobic degradation and incineration [7]. However, stale bread can have other valuable applications, such as producing beer or ethanol. The valorization of sugars from the high starch content is the most promising solution to bread waste, requiring enzymatic hydrolysis through alpha- and glucoamylases [6]. Starch conversion requires enzymatic hydrolysis to obtain glucose and dextrans to rearrange the glucose into glucose molecules [5]. There is an urgent need to transform the present global food systems into healthy and sustainable systems, since the baking industry has a significant impact on the Earth's ecosystems, contributing to the climate change crisis with a large CO₂ footprint. The agricultural industry alone uses around 40% of the planet's fertile land, generates around 30% of greenhouse gas emissions, and uses 70% of the available freshwater [10].

Bread waste can be valorized and upcycled back into the food value chain through its use in beer production, substituting part of the required malt with stale bread [11]. Bread's composition is divided into starch (500–750 g/kg), sugars (3–50 g/kg), and proteins (100–150 g/kg). Bread waste represents a promising substrate for ethanol production due to a predictable yield, reaching 350–370 g/kg of dry matter substrate. Even if spoiled with mold, the yield can reach 240 g/kg [12]. There is great interest in optimizing starch hydrolysis, mainly targeting the liquefaction and saccharification stages, to attain a glucose-rich fermentation source [13]. Studying the maximum stale bread-to-malt mass ratios is important to avoid obstacles while brewing, like issues related to slurry mash, taste defects due to unwanted levels of diacetyl formation, as well as salt and remaining yeast presence in stale bread, with direct impacts on the final beer's flavors [14]. Furthermore, recent studies in this field have shown the intrinsic benefits connected to brewing from bread in 50:50 ratios under a three-step mashing process (preheating at 40 °C/20 min; enzymatic activity at 67 °C/120 min; and a final rest at 78 °C/10 min), underlining potential health benefits not found in regular beer [15].

Focusing on the optimization of bread waste incorporation into beer production reflects two potentially great initiatives towards a healthier world. First, reducing food waste means avoiding nutrient loss, optimizing the human, energy, and economic efforts applied in the food production workflow [7,16], and reducing gas emissions from waste degradation. Regarding global warming concerns, brewing with stale bread corresponds to savings of −46 kg CO₂ eq [17]. Second, barley is a rich grain capable of contributing to human caloric intake on a daily basis [18], so saving the grain to produce cereal-based products would help to provide richer diets and help fight against a recent crisis in the cereal world trade [19,20].

This work shares the goals of the Circular Economy Action Plan, from sustainability to circularity, by giving a second life to a worldwide food staple that eventually ends in waste—bread. Aside from the undeniable environmental impact associated with the conjecture of bread waste circularity and sustainable brewing, processing bread waste as a raw material will impact food general cost reduction [21]. In other words, brewing from stale bread may set an example of a positive socioeconomic impact, reducing food waste.

2. Materials and Methods

2.1. Production and Materials

2.1.1. Equipment

The mashing step was performed in a Braumeister 20 L brew system (Speidel, Of-terdingen, Germany) to escalate a micro-trial experiment to industrial production easily. Fermentation took place in a fermentation bucket (white polypropylene plastic, 30 L) with an airlock and a spigot, extra conditioned with Bemis PM-996 wrapping film (Neenah, WI, USA) to prevent air leaks. An immersion wort chiller was used for a clear cold break at the end of production. The bottling step was carried out with sterilized reused glass beer bottles.

2.1.2. Ingredients

The process followed the main components presented in the disclosed Pale Ale Homebrew Recipe shared by Toast Ale, intended for 28% stale bread incorporation [22]. Some adjustments in quantities were made to attain the set initial goal, a beer brewed mainly from bread. For this purpose, malt replacement by stale bread was calculated in a 1.4 ratio. Stale bread was supplied by a regional bakery established in Santarém, produced with T65 + T85 flours. Also, water is a key component in the brewing industry, mainly for its alkalinity and pH [23]. The water used has a pH of 5.4, providing the optimal enzymatic environment within the range of pH 5.2–5.4 [24]. Relevant composition related to minerals reveals concentrations of Ca^{2+} 0.7 ± 0.2 ppm, Mg^{2+} 1.7 ± 0.2 ppm, SO_4^{2-} 1.5 ± 0.2 ppm, and HCO_3 12 ± 4 ppm.

2.1.3. Process

The bread was transformed into 1 cm^2 cubes and underwent temperature pretreatment in the oven to dry at $90 \text{ }^\circ\text{C}/1 \text{ h}$. The brew system was filled with 20 L of water, and its temperature was raised to $45 \text{ }^\circ\text{C}$ in order to add both malt and bread, immediately starting the beta-glucanase stage for 120 min, followed by the peptidase stage at $50 \text{ }^\circ\text{C}/10 \text{ min}$, the protease stage at $55 \text{ }^\circ\text{C}/10 \text{ min}$, the beta-amylase stage at $60 \text{ }^\circ\text{C}/45 \text{ min}$ and $65 \text{ }^\circ\text{C}/45 \text{ min}$, the alpha-amylase stage at $70 \text{ }^\circ\text{C}/60 \text{ min}$; then, a final rest to stop enzymatic activity was performed at $75 \text{ }^\circ\text{C}/5 \text{ min}$. After a sparging step at $78 \text{ }^\circ\text{C}$ to further extract the remaining sugars, the grain and bread mix were removed from the brew system, allowing us to proceed with the boiling step at $100 \text{ }^\circ\text{C}/60 \text{ min}$. In this stage, hop addition was studied to balance α -acid and β -acid composition [25] for each type used, to achieve the final expected flavors in a pale ale beer. The time window and quantity addition followed Table 1.

Table 1. Hop type, quantity, and time addition during the boiling step of pale ale brewed with stale bread.

Addition Time Window during BOILING	Hops (α -Acids Content)		
	Bramling Cross (6.80%)	Cascade (5.75%)	Centennial (9%)
0 min	0 g	20 g	5 g
15 min	20 g	0 g	5 g
30 min	15 g	10 g	0 g
45 min	0 g	10 g	5 g
60 min	10 g	5 g	0 g

A cold break was performed to decrease wort temperature to $20\text{--}30 \text{ }^\circ\text{C}$ before transferring it to the fermentation vessel and proceeding with yeast addition [26]. Fermentation under a controlled environment was performed at $18 \text{ }^\circ\text{C}$ for 10 days. A priming step was performed to achieve the desirable beer carbonation, by adding 6 g/L of pure glucose to the non-matured beer before bottling. Finally, the aging phase was split into two stages.

First, bottled beer remained isolated from light for 14 days at room temperature. Secondly, bottles were refrigerated at 8 °C for 31 days.

2.2. Analytic Methods

2.2.1. Soluble Solid Content

Soluble solid content (SSC) was analyzed through triplicated samples taken during production in 15 min intervals of the total mashing time. Samples were measured directly in a Hanna—HI96801 digital refractometer (Hanna Instruments, Smithfield, RI, USA) and quantified in °Brix.

2.2.2. pH Measurement

Wort stability was analyzed through triplicated samples taken during production in 15 min intervals of the total mashing time. Samples were measured with a pH electrode connected to a potentiometer (pH92 Lab pH Meter, Radiometer, Copenhagen, Denmark) to determine pH evolution.

2.2.3. Analysis of Volatile Compounds

For a better understanding of the final beer organoleptic profile, the evolution of volatile compounds was analyzed for samples taken during the three main stages: boiling (wort), fermentation (green beer), and aging (final beer). Static headspace sampling was performed with the headspace autosampler, TriPlus RSH System (Thermo Finnigan, San Francisco, CA, USA). A 2.5 mL headspace syringe for the PAL System was used for the injection of 2 mL from the 20 mL headspace vials with 1 g of measured dry sample. The autosampler conditions were set as follows: incubation temperature, 80 °C; incubation time, 10 min; syringe temperature, 100 °C; agitator speed, 500 rpm; fill speed, 100 µL/s; pullup delay, 1 s; injection speed, 500 µL/s; pre- and post-injection delay, 500 ms; flush time, 10 s. After each injection, carryover in the syringe was eliminated by an automatic flush of the syringe with carrier gas. Chromatographic separation was achieved by Thermo Scientific TRACE 1300 gas chromatograph coupled to a Thermo ISQ mass selective detector. A DB-1 (30 m × 0.25 mm i.d.) (Agilent J & W Scientific, Folsom, CA, USA) fused silica capillary column with a 0.25 µm film thickness was used with helium as carrier gas (purity > 99.9997 vol % and flow rate = 1.0 mL min⁻¹). The oven temperature program was started at 60 °C (not held) and a linear temperature gradient was applied at a rate of 3 °C/min to a final temperature of 260 °C and held for 5 min (total run time: 65 min). The ion source temperature was kept at 230 °C, the transfer line was at 150 °C, and the mass spectra were obtained in the 50 to 500 m/z range, at an electron energy of 70 eV [27,28]. The peak areas in the TIC were determined and expressed as normalized relative percentages. The calculated composition was semi-quantitative/qualitative since no standards for each chemical family were co-injected nor were their response factors determined. Each aliquot was injected in triplicate, and the percentage of relevant olfactory compounds was calculated for each sample profile.

2.2.4. Analysis of Sugars and Main Metabolites

The concentration of ethanol, glycerol, organic acids, and sugar was determined by High-Performance Liquid Chromatography (HPLC). Sample clarification was performed by centrifuging samples at 4 °C for 15 min per 16,000 × g, followed by a double dilution, first in a 1:2 proportion in H₂SO₄ 50 mM with centrifugation at 20 °C for 5 min per 16,000 × g, and secondly the supernatant was again diluted in a 1:10 proportion with an equal procedure. The resulting supernatant was filtrated through a cellulose acetate membrane with 0.22 µm pores [29,30].

Ethanol, glycerol, and sugar contents were determined in a Hitachi Refraction 5450 index detector Chromaster UV-Vis Detector 5420 connected to a Hitachi Chromaster HPLC system equipped with an Ionic Exclusion Column (Rezex™ ROA Organic Acid H+ (8%) column, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA), at 65 °C, and sulfuric acid

(5 mM) was used as a mobile phase at a flow of 0.5 mL/min. Total run-time was 40 min. The injection volume was 20 L. Each aliquot was injected in duplicate. Standard samples of each sugar and metabolite (ethanol and glycerol) were used to construct the calibration curves, using the same HPLC parameters. The concentration of each compound in the samples was calculated using the corresponding calibration curve.

2.3. Qualitative Methods

2.3.1. Panel Theoretical Background

A survey regarding background perception around circular economy practices in the food industry and food waste awareness was given to the tasting panel. First, participants were identified by age group, education degree, average beverage consumption, and sensorial analysis experience. Secondly, overall knowledge of circular economy strategies and global food waste volume was elicited [31].

2.3.2. Sensorial Analysis

A survey focusing on the final beer's overall organoleptic character and consumer acceptance was required. The main focus was to avoid an apparent distinction between regular standard beer and the final beer brewed under this project. A panel of 25 participants performed a Triangle Test with 3 samples randomly labeled with a three-digit code [32] to distinguish the beer brewed with stale bread from the standard beer. At a sensorial level, the organoleptic perception was split into seven parameters for more precise feedback about future improvements. Final beer sensorial analysis took into consideration empirical characteristics such as color, foam, aroma, flavor, bitterness, residual palate, and clearness [33]. Each parameter was ranked from 1 (unpleasant) to 5 (great), where the middle value of 3 represented the standard beer profile. Tests were conducted individually under an ingestion temperature range between 12 and 14 °C [34].

2.4. Statistical Analysis

The mean and standard deviation of the experimental data and statistical analysis were conducted by GraphPad Prism software (version 9.0.0). One-way analysis of variance (ANOVA) was performed to identify significant differences between two or three samples with *t*-test or Tukey's test, respectively, with significance levels $\alpha = 0.05$. Differences were considered significant when P-values were less than 0.05.

3. Results and Discussion

Regarding the mashing step, studying enzymatic activity effects in mashing wort is essential to optimize starch hydrolysis while balancing time and temperature control to achieve a desirable extraction of fermentable sugar yield [35]. For a better understanding of sugar extraction efficiency, mashing stages were over-extended to maximum times, respecting the traditional temperature range to acquire a proper environment for optimal enzymatic activity, such as proteolysis and β -glucanase degradation: 45–50 °C, β -amylase: 62 to 65 °C, α -amylase: 70 to 75 °C, and enzymatic inactivation will start at 78 °C [36]. Time over-extension of the original Toast Ale Homebrew Recipe (67 °C/60 min + 100 °C/90 min) [22] allows us to study the maximization of enzymatic processing. In initial trials, we failed to meet the recipe target when forcing an increase in the incorporation rate up to 50% stale bread. This over-extension of two constants (time and temperature) allows a clear knowledge of the effects of the heating aqueous system condition forced into the starch granules, recreating its previous gelatinization process. The gelatinization state transforms starch into a more accessible structure to enzymes, catalyzing its conversion to fermentable sugars and dextrins during mashing [37]. An efficient starch saccharification optimizes fermentable sugar extraction, a key indicator for a successful final yield [38].

3.1. °Brix and pH

Throughout the mashing process, samples were taken to clearly understand sugar availability dynamics and pH changes. As seen in Figure 1, liquefaction and saccharification are promising, achieving a good extraction yield rate.

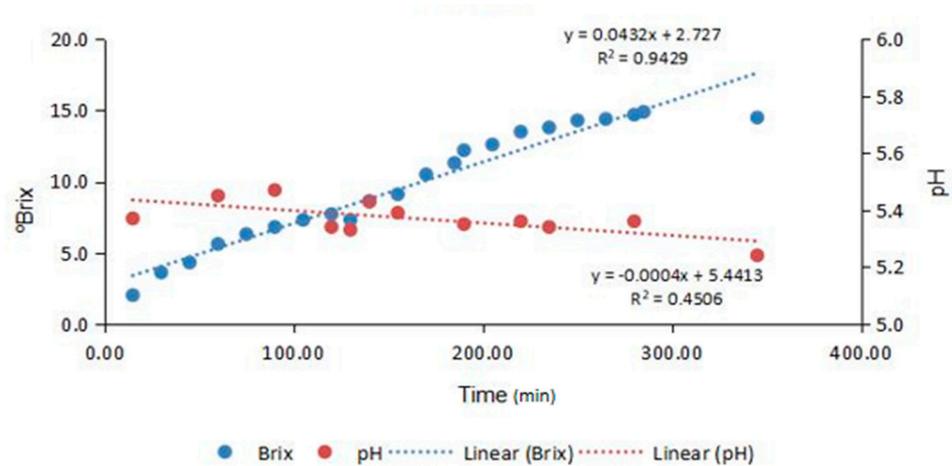


Figure 1. First analysis on °Brix and pH development during pale ale mashing and boiling from a temperature range of 45–100 °C/355 min.

The data analysis confirms that the main factors directly influencing sugar yield in the starch hydrolysis stage are time and temperature, as was observed in previous works focused on ethanol production from bread residues [6]. Regarding pH, stability is essential to ensure food safety standards during public consumption. Moreover, it builds up an ideal acidity environment for proteases to solubilize malt proteins [39] and further amylase activity. Also, pH stability around 5.4 avoids the development of volatile compounds usually associated with unpleasant palate effects related to cardboard- and cabbage-like flavors [40]. After a starting study around the over-extension of time, to validate the theory of saccharide extraction optimization, it was possible to define the optimal process to revert bread starch into available sugars with a technological reapplication based only on time and temperature, with the trial pH subjected to the following analysis.

3.2. Volatile Compounds

Volatile compounds in beer composition are often connected with the human odor sense, from which it is possible to identify certain expected flavors. During fermentation, a secondary chemical reaction pathway generates a wide range of species formed at low concentrations, directly influenced by remaining oxygen, temperature, and pressure [41]. These species correspond to 122 identifiable flavor notes comprising eight odor classes: fruity and floral; grassy and nutty; cereal; caramelized and roasted; phenolic; soapy and rancid; sulfury; oxidized and musty [42]. These classes are combined according to their chemical classification, mainly grouped among acids, alcohols, bases, carbonyl compounds, esters, and sulfurs [42]. Table 2 shows all the data regarding compounds and respective concentrations extracted from GC analysis during beer production stages (boiling, fermentation, and aging). Further analysis could be performed for a more precise characterization of the aged beer. However, these analyses fall out of the scope of this paper, since the main goal is not to brew a distinct product in terms of consumption experience but to brew a product that distinguishes itself by the raw material used to produce it—stale bread. Nonetheless, the main factors that contribute to flavor active compounds are (i) equipment design, (ii) hop composition, (iii) oxygen and carbon dioxide content, (iv) pitching rate, (v) production environment conditions, (vi) raw material composition, (vii) wort specific gravity, and (viii) yeast strain metabolism type [43].

Table 2. Identified volatile compounds by GC in samples of pale ale beer brewed with 50% stale bread, expressed in relative percentage of the normalized peak area in TIC. Same letters in each row represent non-significant differences between the columns according to a *t*-test or a Tukey test ($\alpha = 0.05$).

Compounds	Boiling	Fermentation	Ageing
CO ₂	n.d.	14.01	49.76
Acetaldehyde	n.d.	63.63	n.d.
2,3-Butanediol	n.d.	0.66	n.d.
Acetic acid	n.d.	1.77	n.d.
Oxime-, methoxy-phenyl	n.d.	0.2	n.d.
Linalool	1.39	1.11	3.56
2-Phenylethanol	n.d.	1.02	0.02
Octanoic acid, ethyl ester	n.d.	0.12	5.57
β -citronelool	n.d.	0.24	0.84
2-Phenyl ethyl acetate	n.d.	0.22	0.25
Nerol	0.4	0.22	0.33
α -Humelene	0.08	0.05	0.02
2-Tert-butyl-4-Isopropyl-5-Methylphenol	0.15	0.7	0.99
Caryophyllene oxide	0.03	0.58	0.42
1-Heptadecanol	0.09	0.23	0.12
Eicosane	n.d.	0.19	0.11
Octadecanoic acid, ethyl ester	n.d.	0.03	n.d.

n.d.: non-detectable.

Examining Table 2 requires understanding the identified compounds' potential influence on the final product flavor.

For example, alcohols such as 2-phenylethanol add a rose-like fragrance. This alcohol is a by-product of amino acid breakdown due to the yeast's nitrogen metabolism [44]. Hop contribution comes in the form of 1-heptadecanol, which adds an aniseed-like fragrance, while hop oils contribute with linalool and β -citronelool, which adds an expected flowery lemon lime fragrance [25]. Regarding acids, some traces of acetic acid and octanoic acid were found, adding a vinegar and caprylic scent, respectively. Both are formed during fermentation due to carbohydrate metabolism in the tricarboxylic acid cycle. Although they may negatively affect the beer with off-flavors at high concentrations, their low-concentration presence contributes to the beer's pH and shelf life [42]. Esters are the major flavor active compounds, despite their small threshold values. The most relevant presence in the analyzed samples is 2-phenylethyl acetate, which adds a honey-fruity flavor commonly connected with apples. The fermentation pressure level is considered the factor that mainly influences and controls ester formation, aside from aeration. The kind of ester formation is directly linked with the yeast strain [44]. Carbonyl compounds, such as aldehydes and ketones, often influence the overall flavor, resulting from yeast reduction reactions during fermentation. Usually, acetaldehyde is one of the most abundant components in wheat bread crumb [45,46], possibly adding a green apple scent to the beer [44]. 2,3 butanedione, also known as diacetyl, adds a butter-rounded mouthfeel [47].

3.3. Saccharide and Fermentation Metabolite Level

Outputs from HPLC-calibrated lines were adjusted to analytical graphics to simplify and better understand data retrieved from the samples. These adjustments mirror overall evolution due to enzymatic activity, dependent on the main factors to achieve the extraction goal, time, and temperature. Prominent brewing enzymes are beta-glucanases and xy-

lanases regarding cellulose, proteases regarding proteins, peptidases that can break down proteins into amino acids, and amylases regarding carbohydrates [48].

3.3.1. Glucanases and Pentases

To extract fermentable sugars from gelatinized starch, it is necessary to perform a pretreatment for enzymatic hydrolysis, a simple and naturally reverse route to improve gluten solubility [49]. Beta-glucanase stands as the crucial phase that totally influences successful yield results before starch saccharification, as represented in Figure 2, with a remarkable increase in saccharide concentration after 90 min at this stage.

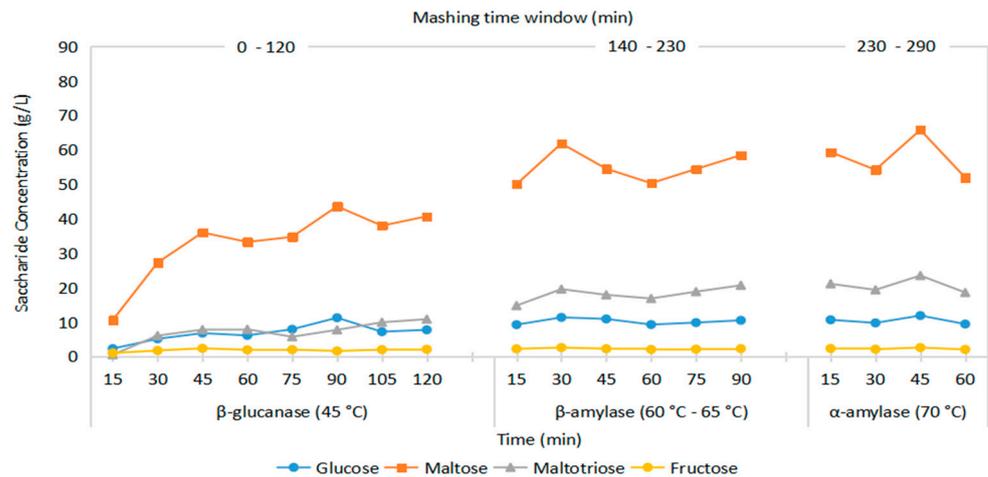


Figure 2. Enzymatic optimal activity analysis during pale ale mashing on the reversal of sugars available in stale bread.

This is the moment when unfermentable sugars will break down and become available again [50]. Also, beta-glucanase overall activity helps lower wort viscosity, and aids in the production of a clear wort, paving the way for further enzymatic activity by digesting the outer layers of starch granules [51]. Although the peptidase and protease phases' main interest is related to protein development, Figure 3 shows a slight increase in sugar content. However, technological interest is mainly pertinent to foam formation and body sharpness [50].

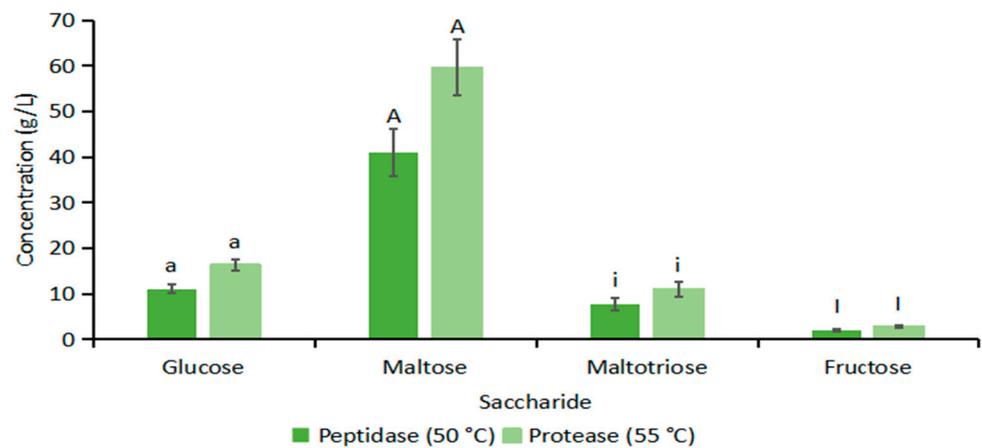


Figure 3. Sugar concentration after the mashing step for peptidase and protease activity at 50 °C/10 min and 55 °C/10 min, respectively, corresponding to the mashing time window of 120–140 min. Sugars in the wort were analyzed by HPLC. Values with the same letters are not significantly different according to a *t*-test ($\alpha = 0.05$).

Protease retains a more important role during mashing, since its activity improves wort clarification by increasing protein solubility degree, fermentation efficiency by facilitating yeast growth with an expanded free amino nitrogen availability, and both storage and chilling quality conservation [51]. Malt proteolytic enzymes find an optimal temperature range around 50–55 °C to produce the essential amido nitrogen originating from the main barley proteins, hordein and glutelin [52]. Most proteolytic malt enzymes show higher activity in alkaline environments, making mashing process temperature management crucial for achieving favorable organoleptic characteristics and time control for avoiding an extensive protein modification that leads to a bread-like flavor [53].

3.3.2. Amylases

Amylases are responsible for breaking starch chains, rearranging them into simple and complex sugars. In terms of optimal activity temperature, alpha and beta amylases differ. However, since enzymatic activity during brewing overlaps, both optimize each other's efficiency. Beta-amylase breaks down starch strands into maltose molecules but only breaks linear 1–4 glucosidic bonds from one end to another in a non-reducing manner [48]. Refocusing on Figure 2, it is possible to understand that beta-amylase has an interesting activity rate in the beginning, although it suddenly decreases due to its own limitation. Nonetheless, extending this stage up to 90 min guarantees a wort rich in maltose, justified by the enzyme activity in a bread substrate that commonly presents maltose as the main saccharide [3]. Gelatinized starch becomes totally available for enzymes, which is to say that diastatic enzymes extract highly fermentable short-chain sugars from starch. This phase also contributes to a crisper and drier feeling in the final beer experience [54]. The overlapping activity comes into play with alpha-amylase, since it also breaks down linear 1–4 glycosidic bonds. Enzymes extract longer-chain sugars and dextrans with less fermentable potential but will directly influence final mouthfeel [54] with extra body. Meanwhile, it digests at any point of the chain, facilitating the limited digestion capacity of beta-amylase while transforming the molecule's linear bonds into an array of smaller fermentable sugars [48]. Comparing both time window optimal enzymatic activities, it is possible to understand that beta-amylase suffers a decrease in its activity rate that again increases when both amylase activities occur in synergy. Amylase activity decrease is expected when over-extended in regular malt mashing [35]. However, over-extension with minimal activity was necessary to better analyze the optimization of sugar extraction from stale bread.

3.3.3. Wort, Green Beer, and Final Beer

Brew wort is abundant in fermentable sugars from malt and bread starch hydrolysis and further enzymatic activity. The main sugars are represented in Figure 4, having glucose consumption priority among *Saccharomyces cerevisiae* strains, even at low concentrations in comparison with maltose, the most abundant sugar, which justifies its residual presence at the end of the fermentation phase, and maltotriose [55].

The full consumption of extracted sugars from stale bread shows a promising use for waste bread as biomass. With a simple pretreatment, it is possible to recirculate at least 85% of its weight into fermentable sugars for ethanol production [8]. *Saccharomyces cerevisiae* digests monosaccharides (glucose and fructose) first, slowing sugar–alcohol conversion rate once it starts digesting disaccharides (maltose) and trisaccharides (maltotriose). The decrease in digestion pace justifies the residual presence of maltose in Figure 4 after fermentation.

A low-alcohol beverage was successfully acquired with the process described. Together with ethanol, a considerable amount of glycerol was also produced, reflecting a partial deviation of glucose from the ethanol fermentation pathway towards the production of this polyol [56]. Nonetheless, glycerol may positively contribute to viscosity and a well-rounded mouthfeel [57]. It restores the thresholds of the stronger aldehyde flavors, being most prominent in ale-type beers [56]. However, to achieve a higher concentration,

tests with high-pressure treatment and optimal fermentation conditions could be carried out to optimize saccharification and achieve higher ethanol concentration [38].

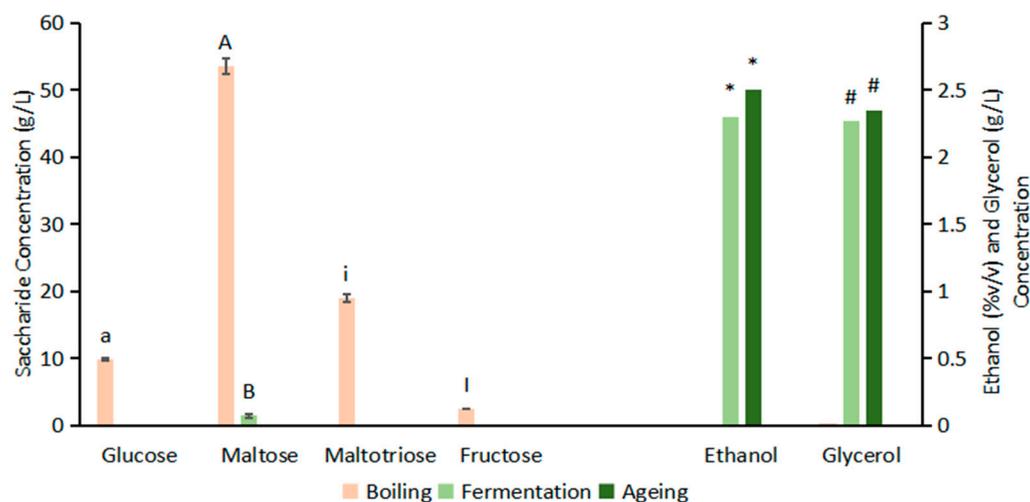


Figure 4. Saccharide concentration in the wort at the end of boiling, after fermentation by *Saccharomyces cerevisiae*, and after the aging phase (left side), and subsequent metabolite concentration (ethanol and glycerol) (right side). Sugars and metabolites in the wort were analyzed by HPLC. Absence of error bars means values too close to 0. Values with the same letters or symbols are not significantly different according to a *t*-test ($\alpha = 0.05$).

3.4. Sensory Analysis

3.4.1. Theoretical Knowledge Data

The age of the volunteers for the tasting panel was spread among different generations grouped in age brackets: 18–25 (10%), 26–35 (26.7%), 36–50 (13.3%), and >51 (50%). More than half of the participants had an academic degree (56.7%). However, more than 3/4 of the participants had no relevant experience in sensory and tasting analysis (76.7%). Due to equipment limitations, an ale-type beer was produced. However, the Triangle test comparison was performed with a standard and commonly consumed Portuguese lager-type beer (Sagres). As expected, the vast majority recognized the different beer by the style/hops (80%), differentiating them at the flavor and aroma level. Despite only 2/3 of the participants being aware of sustainable and circular economy practices in the food industry, almost everyone would support upcoming strategies and ideas (96%). Conscious consumption sensitization may guide the final consumer towards pro-environmental behavior under the domain of food and beverage choices [58]. Beer consumption is a common practice for the majority, drinking beer at least once per week (68%), although less than 1/4 were aware of bread waste volumes and overall impact (24%). Optimistic prospects reappear with a solid acknowledgment of the idea to brew from surplus bread (96%) and a future intention to consume sustainable beer (92%). For most of the tasters (88%), no bread scent was noticed in the beer-tasting analysis. Sustainable beer merely corresponds to the environmental aspects of the commodity, having no human health impact or functional quality impact on the environment. Nevertheless, recent studies reveal an optimistic prospect regarding willingness to purchase sustainable beers [59].

3.4.2. Sensory Analysis Feedback

At first glance, participants were not able to detect any noticeable difference in the beer under analysis. This means that adding surplus bread to beer has a minor impact on the final expected quality of the consumer experience. Previous studies in this field pointed out the possible influence of salt among incorporations above 30% [60], an inconvenience which the present study did not face, probably because of the different chemical composition of the water, or the regulation by law of the total amount of salt in baked goods as low as 1.4%

(*w/w*) of sodium chloride in bread. Also, as seen in Figure 5, some parameters of the final beer character distinguish it from the standard beer, mainly the aromatic profile, due to hop types and addition technique, the clearness (acquired through a cold break after the boiling step), the color (strongly influenced by the malt and bread used), and the foam (optimized during protease optimal time during mashing). However, it is important to underline once more that the volunteers belong to a more disseminated lager beer consumption culture, which may subjectively impact in the comparison degrees.

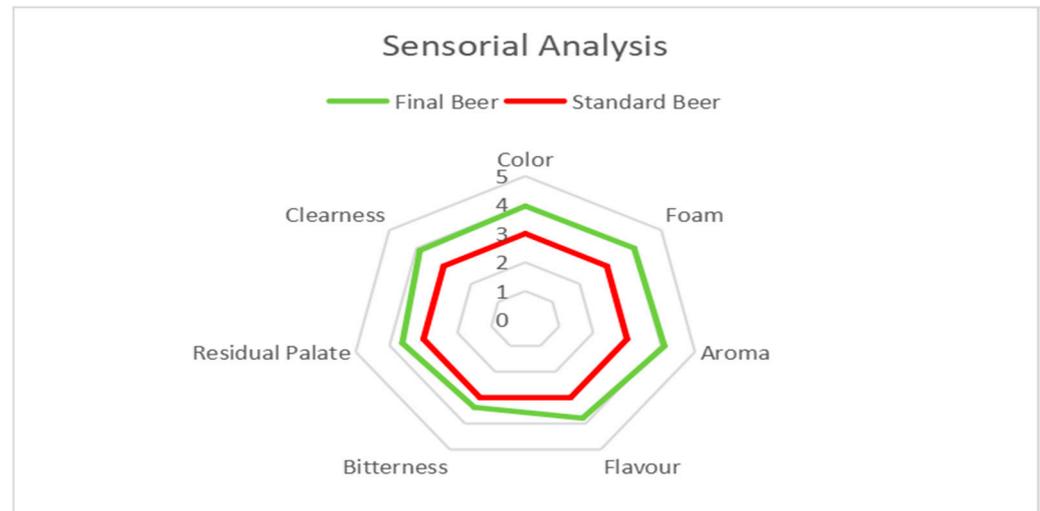


Figure 5. Tasting parameters and results of the hedonistic review on aged pale ale beer with 50% stale bread incorporation.

The rest of the beer character traces revealed a standard profile, solidly achieving the purpose of this study: acquiring a sustainable beer that easily can integrate into the regular beer market without any need to appeal to new consumer niches commonly associated with the craft beer revolution [61].

4. Final Remarks

This bread waste valorization directly impacts food system domains such as sustainable food by upcycling stale bread components, consumer health through a slight decrease in alcohol concentration present in beer, consumer conscientization trends driven by a novel highly sustainable beverage, contributing directly to the mitigation of food waste [62,63]. There are six areas where brewers should look to produce a sustainable beer: beer ingredients, containers and packaging, energy and climate, solid wastes, spent grains, and water conservation [64]. Sustainable beer seems a pleasant option to the final consumer, as the food and beer gastronomy pairing approach, united with a low alcohol content factor, grows around consumers' interest [65]. Beer consumption is increasing in Europe, so the main motivation is a clear symbiosis between the product-related factors and the consumption context-related factors [66]. A green economy case may be accomplished in collaboration. Macro-breweries and bakeries can mimic the micro-brewery interchange of empirical experience and a new hypothesis [67]. The present study was eager to address the traditional brewing process, avoiding the addition of commercial enzymes in order to facilitate the process, as these are considerably expensive. However, most studies addressed this issue by using unconventional alternatives to malt. Commercial enzymes may help macro-scalability with extra sensory additions (ficin and papain) [51] or with the production process (Hitempase 2XL; Bioprotease N-100L; Bioferm L, Fungal Protease FP ISE 8467; Bioprotease P Concentrate) [68]. From a technological point of view, the outcomes show the viability of producing low-alcohol-content beverages through the traditional brewing methods, by replacing malt with stale bread up to 50%. Further analyses are relevant under the scope of food production legislation since, in Portugal, a beer needs

“at least 50% of malt in the total amount of sugars source ingredients” [69]. Ultimately, once the beer industry steps forward to brew from bread as well, more analysis on the scope of nutritional and functionality aspects should be considered in comparison with the discussed advantages associated with the centennial consumption of this kind of beverage, such as Kvass [70].

5. Conclusions

Achieving a beer produced mainly from stale bread requires 185 min for the mashing time only, to fully optimize sugar extraction while avoiding incremental changes, and a whole new perspective on the standard production workflow. From a critical point of view, it may reflect two interesting outcomes: a green economy product and a low-alcohol-content beverage. Brewing mainly from bread waste could result in a novel beverage. Hopefully, it is not only possible, but also viable, to practice the circular economy in the beer industry, creating a new disposable model between farms, bakeries, and breweries.

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