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ORIGINAL ARTICLE

Functionality and economic feasibility of enzymatically hydrolyzed waste bread as a sugar replacer in wheat bread making

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Abstract

Food-grade enzymes (α-amylase, amyloglucosidase, maltogenic amylase, and protease) were investigated for recycling waste bread back to wheat bread-making process. Waste bread was efficiently hydrolyzed into sugars (up to 93% glucose yield) and the best combination of enzymes was α-amylase (0.05 g/kg bread) and amyloglucosidase (2.5 g/kg bread). Selected enzyme hydrolysis processes were tested in wheat bread making as a (a) hydrolyzed slurry, that is hydrolyzed waste bread without solid/liquid separation and (b) syrup, that is liquid supernatant after centrifugation of the hydrolyzed waste bread. Both hydrolyzed bread slurry and syrup were successfully utilized to replace sucrose (2 and 4%) in bread making without affecting the bread quality when compared to the control bread. Techno-economic assessment revealed that this approach is 12% more economical than the current mean to dispose the bakery waste. This recycling concept showed both technical and economic potential for bakery industries to overcome their excess bread production.

Novelty impact statement: A new recycling process was developed by using enzymes to efficiently hydrolyze surplus bread into sugars. The use of those sugar-rich slurries and syrups in bread rework did not affect the bread quality when compared to the control bread. The recycling concept was more economical than current means to dispose waste bread, revealing the technical and economic potential for bakery industries to overcome their excess bread production.

1 | INTRODUCTION

Bread is an important staple food consumed worldwide. However, bread is also among the major food waste in many countries around the world. Annual global production of bread exceeds 100 million tons and estimated wastage for bakery goods is about 7–10% (Melikoglu & Webb, 2013; Mena et al., 2011), implying a substantial amount of food escaping from human nutrition. During the bakery process, waste is produced from overproduction of bread, excess dough, dusting flour, and from defective products that randomly occur during the production line. Current means to deal with bakery waste involves incineration, utilization as animal feed, or biofuel production, whereas efficient recycling back to food industry is nonexisting. Research has been done to develop strategies for waste bread

following a biorefinery approach to produce ethanol, lactic acid, succinic acid, and glucose-rich syrup (Haroon et al., 2016; Leung et al., 2012; Pietrzak & Kawa-Rygielska, 2015). However, reports on recycling waste bread back to bread-making process are rare.

Due to the composition of bakery waste, that is mainly starch, waste bread is an interesting raw material for the production of sugar-rich ingredients such as glucose syrup. In the conventional glucose syrup production, starch (mainly from wheat, corn and potato) is converted into glucose in three stages: gelatinization (dissolution of starch granules to form a viscous suspension), liquefaction (partial hydrolysis of the starch), and saccharification (production of glucose and maltose by further hydrolysis) (Olsen, 1995). Different amylolytic enzymes used in these processes are as follows: (a) endoamylases, such as α-amylase (EC 3.2.1.1) which forms maltooligosaccharides with varying length and α-limit dextrins; and (b) exoamylases, such as amyloglucosidase or glucoamylase (EC 3.2.1.3), which cleaves both α-1-4 and α-1-6 glycosidic bonds producing glucose, and β-amylases (EC 3.2.1.2) that cleave exclusively α-1-4 glycosidic bonds producing maltose and β-limit dextrin (Van Der Maarel et al., 2002). The conversion of bakery waste into glucose by hydrolysis has been previously studied, and for example Melikoglu and Webb (2013) reviewed patents describing enzymatic but also fermentation processes with diverse range of enzymes (cellulases, proteases, amylases, and lipases) to hydrolyze waste bread using diverse apparatus for the incubation. In the scientific literature, few works describe the hydrolysis of waste bread for different purposes. Hudečková et al. (2017) optimized waste bread hydrolysis in order to improve the following fermentation effectivity. Sükrü Demirci et al. (2017) used a response surface methodology to find the optimum substrate, water and enzyme ratio to produce the highest amount of fermentable sugars. Benabda et al. (2018) hydrolyzed powdered waste bread as growth medium for baker yeast. Even though the possible use of waste bread in sugar-rich syrup production has been earlier attempted, the final products were generally for non-food uses and no reports on the utilization of these products or syrups in food production or recycling to bread-making process were found.

Sugar syrups are used in bread making as a source of fermentable carbohydrates for yeast and flavor, as well as crust color formation due to Maillard reactions. Furthermore, the syrups extend the shelf-life of bakery products by increasing hygroscopicity and tenderizing of bread crumb. Sugar composition of the syrup affects the fermentation and Maillard reaction rate (Zargaraan et al., 2016), and thus, optimizing the sugar composition in syrup production is important for the bread quality.

The aim of this work was to investigate whether a syrup prepared from enzymatically hydrolyzed waste bread can be used as a sugar replacement ingredient in wheat bread making. For this purpose, food-grade amylolytic enzymes were used to hydrolyze starch from waste wheat bread into glucose or maltose. Protease was also added into the system to study the impact of protein hydrolysis on the starch hydrolysis efficiency. The hydrolyzed waste bread slurry as well as its syrup (liquid supernatant separated from the slurry by centrifugation) were used to replace 2 or 4% of sucrose during bread making, and evaluated by measuring bread volume and texture. Furthermore, a techno-economic assessment (TEA) was conducted to evaluate the economic feasibility of recycling the waste wheat bread inside a small-scale theoretical bakery.

2 | MATERIAL AND METHODS

2.1 | Raw materials

Waste wheat bread (*French country bread*) was obtained from a local bakery (Sinuhe). Waste bread was composed of breads produced in excess or the ones that did not reach standard of weight or appearance. They were collected directly after baking and frozen for further experiments. Waste bread was ground with a cutting mill with 1 cm × 1 cm sieve. For the hydrolysis in large scale (item 2.2.2), the waste bread was just sliced (around 1 cm) with a knife. Waste bread was characterized for total protein content by the American Association of Cereal Chemists (AACC) method no. 46-11A, fat by AOAC method no. 922.06, starch by the Megazyme assay kit (method 76–13.01), and ash gravimetrically by burning at 550°C. The moisture content in raw material was determined by air-drying following by oven-drying (overnight, 105°C) to constant mass. All analyses were performed in duplicate.

Amylolytic enzymes α-amylase (Grindamyl A14000) (ALPHA) and amyloglucosidase (Grindamyl PlusSweet) (AMYL) were kindly provided by Dupont. Maltogenic amylase (MALT) was kindly provided by Bio-Cat. Protease (Corolase 7089) (PROT) was purchased from AB-Enzymes GmbH. The enzyme dosages were based on ranges recommended by suppliers and/or previous screening trials. The enzyme activity of α-amylase was 129 k CU/g (CU = Ceralpha Unit, one unit of activity is defined as the amount of enzyme required to release one micromole of p-nitrophenol from BPNPG7 in 1 min) according to Megazyme standard method for determination of α-amylase (K-CERA 08/16). The enzyme activity of amyloglucosidase was 541,942 nkat/g measured according to Bailey and Pessa (1990). The enzyme activity of protease was 8476 nkat/ml measured according to Matsubara et al. (1958). The materials used for the experimental baking included wheat flour (Helsingin Mylly), yeast (Suomen Hiiva), sucrose (Suomen Sokeri), salt (Meira), and fat (Sunnuntai, Raisio Oyj).

2.2 | Enzymatic hydrolysis conditions

2.2.1 | Small scale

Enzyme solutions were prepared by dispersing determined amounts in distilled water using a magnetic stirrer (50°C in a water bath). Enzyme dosages varied between 0.001–0.05 g/kg bread for α-amylase, 0.25–2.5 g/kg bread for amyloglucosidase and maltogenic amylase, and 0–8.4 g/kg bread for protease. Ground waste bread was weighted in Falcon tubes and enzyme solutions were added

on it. The ratio in weight for the slurry was 1:2 for bread : water. The tube was well shaken in order to wet the entire sample. As a blank sample, bread was mixed only with distilled water (no enzymes added). Samples were incubated at 50°C for 24 hr. At the end of incubation time, the tubes were centrifuged (10 min, 10,000*g*, 22°C) and the supernatant was collected in a glass tube, which was put in boiling water for 10 min to inactivate the enzyme. Thereafter, the sample was cooled down in ice and stored in the freezer for posterior characterization.

2.2.2 | Large scale

Based on the results of small-scale hydrolysis, three enzyme dosages were selected (Table 1) and prepared in large scale to be used in baking. In the large-scale experiment, waste bread was sliced and the enzyme solutions prepared similarly as in the small scale. The bread slurry was prepared at 1:2 bread water ratio, and incubation was performed at 50°C for 24 hr without any mixing step. Enzymes were inactivated by boiling the slurry for 10 min. The hydrolyzed slurry samples were cooled down and frozen. The hydrolysis process was replicated but with a centrifugation step (15 min at 4,000*g*) in the end to remove the solids from the bread slurry. The supernatant was collected, cooled down, frozen, and named as a "*syrup*." Both samples, that is hydrolyzed slurry and syrup, were used for baking applications.

2.3 | Characterization of the hydrolyzed slurries and syrups

Reducing sugars present in the supernatant of waste bread after enzymatic hydrolysis were quantified by the DNS method (Miller, 1959) ($n = 3$) using glucose as a standard. Free nitrogen content was measured by using a FLASH 2000 series analyzer ($n = 3$) as described in Nordlund et al. (2018). Free sugar profile was analyzed from all syrups and hydrolyzed slurries ($n = 2$) using HPAEC-PAD chromatography according to Xu et al. (2017). The HPAEC-PAD system used for the analysis contained an autosampler (Waters 717 plus), three HPLC pumps (Waters 515), Dionex CarboPac PA1 Guard Column and Analytical Column (Thermo Scientific), and an electrochemical detector (Waters 2465).

2.4 | Experimental baking procedure

Wheat breads were baked using both the hydrolyzed slurries and the syrups from waste bread. These samples (hydrolyzed slurries and syrups) were used at an addition level ranging between 9.7–20.4% flour weight for 2% sugar replacement and 18.0–32.3% flour weight for 4% sugar replacement, calculated based on their free mono- and disaccharide content (maintaining the 2 or 4% sugar content in all dough types). These samples replaced dough sucrose, part of flour, and water keeping the dough yield and dry mater-water ratio the same. The compositions of bread doughs at 2% sugar content are shown in the Table 2.

Control wheat bread was prepared in each baking session and, therefore, results were derived as an average from all control breads. All other dough types were prepared as two replicate baking procedures. Bread ingredients (flour, water, bread slurry/syrup, sugar, salt, yeast, and fat) were mixed 3 min slowly and 4 min fast with a spiral mixer (Diosna Dierks & Söhne GmbH), followed by a 15-min floor time in the fermentation cabinet (35°C, 75% relative humidity, Lillnord). The dough was then divided into loaves (250 g each) and molded mechanically, then proved for 45 min in the fermentation cabinet (35°C, 75% relative humidity). For breads containing maltose hydrolysate or maltose syrup, 55 min proofing time was used instead of 45 min. The breads were baked in 200°C oven (Sveba Dahlen) for 15 min with 15 s of steaming in the beginning. After 1 hr of cooling, the loaves were weighed, packed into plastic bags, and stored in room temperature.

TABLE 1 Enzyme dosages and sugar profile of samples selected to be used in the baking recipes

	Enzyme dosages (g/kg bread)				Sugar profile (mg/g)	
Samples	α-Amylase	Amyloglucosidase	Maltogenic amylase	Protease	Glucose	Maltose
Hydrolyzed slurry						
Blank Slurry (no enzymes)	–	–	–	–	0.2 ± 0.0	7.1 ± 0.5
ALPHA-AMYL	0.05	2.5	–	–	192 ± 5	<DL
ALPHA-MALT	0.05	–	2.5	–	3.3 ± 0.3	131 ± 10
ALPHA-AMYL-PROT	0.05	2.5	–	4.6	195 ± 9	<DL
Syrup						
ALPHA-AMYL	0.05	2.5	–	–	222 ± 2	<DL
ALPHA-MALT	0.05	–	2.5	–	2.7 ± 0.1	121 ± 2
ALPHA-AMYL-PROT	0.05	2.5	–	4.6	211 ± 1	<DL

Abbreviations: <DL, under detection limit; ALPHA-AMYL, α-amylase and amyloglucosidase; ALPHA-AMYL-PROT, α-amylase, amyloglucosidase, and protease; ALPHA-MALT, α-amylase and maltogenic amylase.

TABLE 2 Baking recipes for control bread and breads with added hydrolyzed slurry or syrup representing 2% per flour weight (% f.w.) sugar replacement

		Hydrolyzed slurry			Syrup		
Ingredients (% f.w.)	Control bread	ALPHA-AMYL	ALPHA-MALT	ALPHA-AMYL-PROT	ALPHA-AMYL	ALPHA-MALT	ALPHA-AMYL-PROT
Flour	100.0	98.7*	95.7*	98.9*	99.6*	97.7*	99.6*
Water	60.3	52.6	46.2	53.2	53.0	47.3	53.3
Salt	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sugar	2.0	—	—	—	—	—	—
Hydrolyzed slurry or syrup	—	11.0	20.4	10.2	9.7	17.2	9.5
Yeast	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Fat	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Total	174.8	174.8	174.8	174.8	174.8	174.8	174.8

Abbreviations: ALPHA-AMYL, α-amylase and amyloglucosidase (0.05 and 2.5 g/kg bread, respectively); ALPHA-AMYL-PROT, α-amylase, amyloglucosidase, and protease (0.05, 2.5, and 4.6 g/kg bread, respectively) ALPHA-MALT, α-amylase and maltogenic amylase (0.05 and 2.5 g/kg bread, respectively).

*The portion of flour was substituted with the non-sugar dry matter from hydrolyzed slurry or syrup.

2.5 | Characterization of new breads

New breads were characterized using specific volume (SV) and crumb hardness as quality measures. Springiness and resilience of test breads were followed along the test bakings. BreadVolscan laser scanner (Backaldrin, Austria) was used to analyze the SV of three replicate breads during the day after baking. Texture analyzer (TA-XT2i, Stable Micro Systems Ltd.) was used for the texture profile analysis of all breads according to Wang et al. (2019). Crumb hardness (g) was measured after bread storage at room temperature for 1 and 4 days.

2.6 | Statistical analysis

Statistical analysis for experimental baking results was carried out with SPSS Statistics 25 software (IBM Corp.) by one-way analysis of variance (ANOVA) and Tukey's post hoc test using $p < .05$.

2.7 | Techno-economic assessment

For evaluating the feasibility of the waste wheat bread recycling process concept, a conceptual-level TEA was conducted for a theoretical small-scale bakery having a wheat bread production line capacity of 1000 t flour/a (baked bread 3.7 t/d). The total bakery waste amount was assumed to be 10% of the baked bread amount (0.37 t/d), from which 80% consists of off-specification baked bread and the rest 20% of raw dough.

In the TEA, two concepts were evaluated and compared. In the Reference concept, the waste wheat bread and raw dough are disposed to bioethanol production, which is one of the current means of Finnish bakeries to dispose their wastes. In the Recycling concept, all of the baked waste bread is hydrolyzed. Due to the activity of yeast in the raw dough, it cannot be hydrolyzed. Instead, it is disposed to bioethanol production as in the Reference concept. The hydrolyzed bread slurry is recycled without solid separation to bread dough preparation to substitute sugar, water, flour, salt, and preservative in the dough. The following dough recipe was used in both concepts: wheat flour 100% per flour weight (% f.w.), water 60.3% f.w., emulgator 1% f.w., salt 1.5% f.w., and preservative 0.05 wt.% in product. The sugar and yeast contents varied. In the Reference concept, they were 2% f.w. and 5% f.w., respectively, and the sugar was introduced to dough via glucose syrup. The baking trials showed that when recycling the hydrolyzed slurry, the sugar content may be increased to max 5% f.w. without affecting the quality of the final product. This additional sugar amount will decrease the yeast dosage by 10% from the reference recipe to 4.5% f.w.

Mass and energy balances were calculated using a steady-state simulation software, Balas®. The simulated process consisted of dough preparation, proving at 35°C, a gas-fired baking oven with subsequent cooling of the baked bread to 25°C, a hydrolysis reactor followed with enzyme inactivation at 90°C and cooling to 45°C, and the recycling of the hydrolyzed slurry to dough preparation. The process parameters and yields in the hydrolysis were based on the scale-up experiments, with the enzyme dosages of 0.05 g/kg bread for α-amylase and 2.5 g/kg bread for amyloglucosidase, a bread:water ratio of 1:2, and incubation temperature and time of 45°C and 12 hr, respectively. The inactivation of yeast, emulgator, and enzymes during baking, as well as the partial evaporation of preservative during baking and hydrolysis were also considered in the model. The energy balance calculation was less emphasized. Only the total specific energy consumption in the oven was included: 3.1 MJ/kg baked bread, from which the share of gas is 95%, the rest being electricity. Heating, cooling or mixing duties in dough preparation, proving, hydrolysis, and enzyme inactivation were excluded. Figure 1 depicts the block diagrams for the Reference and Recycling concepts.

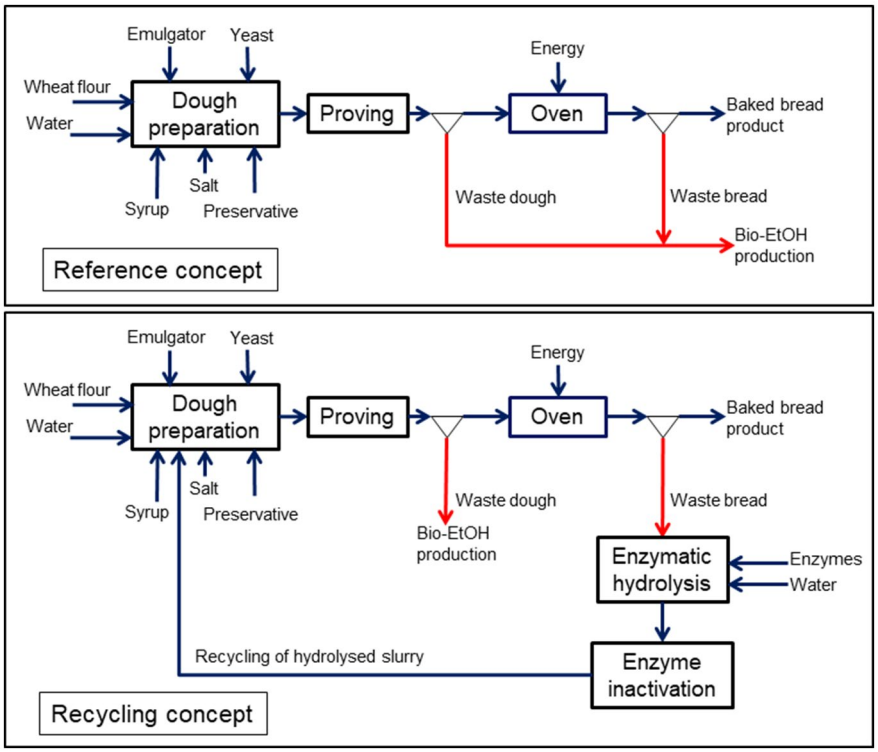

FIGURE 1 Block diagrams for the current mean (Reference) and the new mean to treat bakery waste (Recycling)

For evaluating and comparing the economic viability of the alternative concepts for treating the bakery waste, variable costs and fixed costs were defined. Only those variable and fixed cost items were included that were somehow affected by the implementation of the recycling concept. Variable costs included raw materials, utilities, and wastes. The additional fixed costs, derived from the implementation of the hydrolysis step and valid only for the Recycling concept, included maintenance (1.5% of the fixed capital investment [FCI]), taxes and insurances (1% of FCI), and capital charges. The capital charges were calculated by annualizing the FCI with a 10-year lifetime, 86% plant utilization degree, and 5% interest rate resulting in an annual charge capital ratio (ACCR) of 13%. Labor costs were not included in either of the concepts, because it was assumed that the existing bakery staff operate the hydrolysis step.

The overall focus in the Recycling concept was to maximize the utilization of existing equipment and resources available at the bakery site and to minimize new equipment purchases. For the Reference concept, no new investments are required. In the Recycling concept, the waste wheat bread is hydrolyzed batchwise in a hydrolysis reactor (1.2 m^3). A buffer tank (2.4 m^3) after the reactor is needed to buffer the flow between hydrolysis and dough preparation. The reactor and the buffer tank are assumed to be purchased as used equipments. The purchased equipment cost (PEC) estimate for used reactors and tanks was based on a rough estimate, 1 €/L volume, suggested by bakery industry representatives. The FCI was estimated using a factor of 1.6 (Lang factor) as the ratio of FCI to the sum of PEC, as suggested for food industry by Marouli and Maroulis (2005).

Finally, the total production costs (€/a and €/t bread) of the novel approach for treating the bakery waste inside the bakery were calculated and compared with the cost derived from the current mean of disposing the bakery waste. A sensitivity analysis was performed to reveal which input parameters are both uncertain and influence the total production cost.

3 | RESULTS

3.1 | Enzymatic hydrolysis of waste bread

3.1.1 | Effect of amylolytic enzymes and protease on sugar release (small-scale hydrolysis)

The main composition of waste bread is as follows: 70.5, 14.1, 2.7, and 0.8 g of starch, protein, ash, and lipids, respectively, per 100 g d.m. When waste bread was treated with water without enzymes (blank), 12 mg of reducing sugars were released per ml of liquid supernatant. The addition of α-amylase increased this amount to 40–80 mg/ml based on the dosage of enzyme used (0.005–0.05 g/kg bread) (Figure 2a). The combination of α-amylase and amyloglucosidase was the most efficient way to hydrolyze starch into reducing sugars reaching up to 178 mg/ml with the highest doses of enzyme (Figure 2a). This was equivalent to a glucose yield of 93% (assuming that 1.0 g of starch converts to 1.11 g of glucose and that the reducing sugars are mainly glucose). When comparing the influence of α-amylase and amyloglucosidase on starch hydrolysis, the addition of amyloglucosidase overrun the effect of α-amylase. For example, at

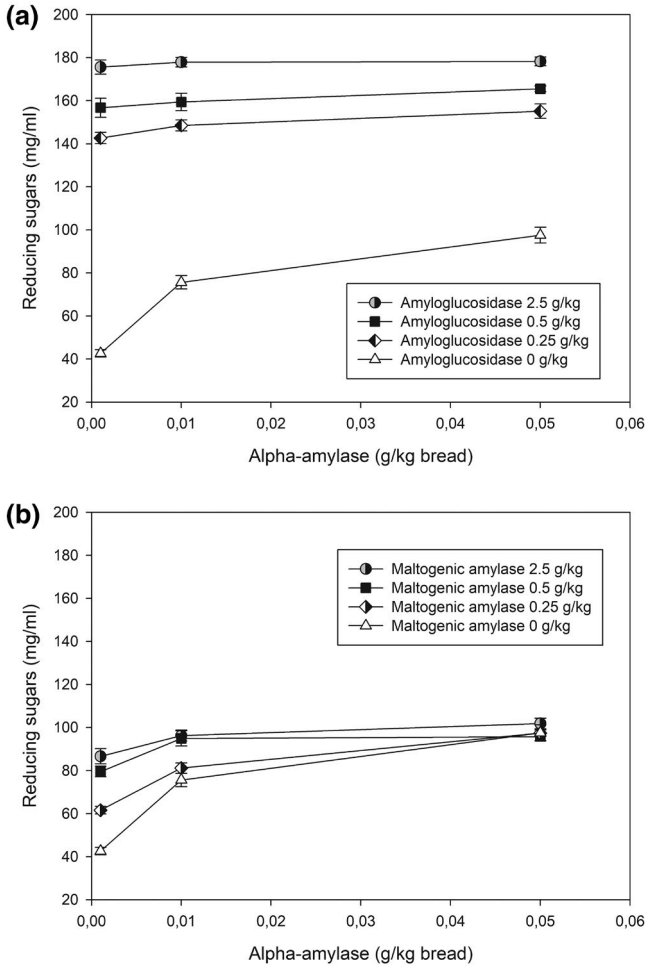

FIGURE 2 Release of reducing sugars (mg/ml) from waste bread treated with different dosage of α-amylase (0.001–0.05 g/kg bread) combined with either (a) amyloglucosidase (0.25–2.5 g/kg bread) or (b) maltogenic amylase (0.25–2.5 g/kg bread)

the highest dosage of amyloglucosidase (2.5 g/kg bread), the amount of reducing sugar released was independent of the α-amylase dosage, that is same amount of sugar was released when adding either 0.005 or 0.05 g of α-amylase per kg bread (Figure 2a).

Comparing exoamylases action, maltogenic amylase combined with α-amylase (Figure 2b) was much less efficient to release reducing sugars compared to amyloglucosidase. When maltogenic amylase was combined with the highest dosage of α-amylase (0.05 g/kg bread), no dose–response of maltogenic amylase was observed. In fact, maltogenic amylase did not release more sugars than α-amylase itself at its highest dosage (0.05 g/kg bread) (Figure 2b). A dose–response of maltogenic amylase action was observed only when α-amylase was dosed at lower levels (Figure 2b).

When the effect of protease addition on sugar release was studied, α-amylase dosage of 0.05 g/kg bread and amyloglucosidase dosage of 2.5 g/kg bread were selected and combined with different dosages of protease (0.05, 0.5, 4.6, and 8.4 g/kg bread). The addition of protease did not improve the starch hydrolysis in any of the enzymatic combinations (Figure 3). On the contrary, the addition of

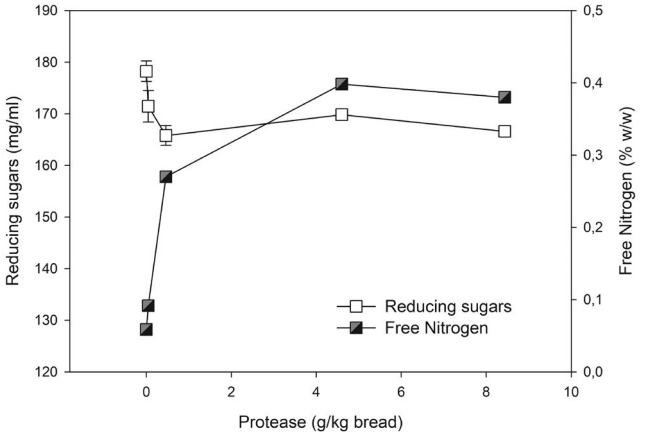

FIGURE 3 Release of reducing sugars (mg/ml) and free nitrogen (% wt/wt) from wheat bread treated with α-amylase (0.05 g/kg bread), amyloglucosidase (2.5 g/kg bread), and different dosages of protease (0–8.4 g/kg bread)

protease slightly reduced the amount of sugars released (4–7% reduction) when compared to the sample treated only with α-amylase and amyloglucosidase. As expected, the addition of protease released free nitrogen in the system (from 0.06 up to 0.4% wt/wt), and this increase was concomitant to the level of protease added (Figure 3).

3.1.2 | Sugar profile of waste bread after enzymatic hydrolysis

Based on the results shown above, three different conditions were selected (Table 1) and the hydrolysis in large scale was performed for further use in baking. The composition of sugars (glucose and maltose) in these samples were analyzed and shown in Table 1. The blank sample presented only traces of glucose and maltose. Both the hydrolyzed slurry and syrup samples from the waste bread treated with α-amylase and amyloglucosidase (ALPHA-AMYL) mainly contained glucose, as expected (192 and 222 mg/g, respectively). These values were equivalent to a glucose yield of 100 and 115%, respectively. These values were higher than the yield in the small-scale trial (93%) which was likely due to some evaporation of water during the boiling step (the evaporation was not counted in the calculation of the yield because it could not be measured). Maltose was the main sugar in the hydrolyzed slurry and syrup samples (131 and 121 mg/g, respectively) when the waste bread was treated with maltogenic amylase (ALPHA-MALT). The hydrolyzed slurry and syrup treated with α-amylase, amyloglucosidase, and protease (ALPHA-AMYL-PROT) had a similar sugar profile to the corresponding sample without protease (Table 1).

3.2 | Production of bread using syrups and hydrolyzed slurries

The control bread baked without sucrose was harder than the controls with 2 or 4% sucrose (Table 3). The bread containing the slurry

TABLE 3 Hardness (g) and specific volume (ml/g) of breads baked with either hydrolyzed slurry or syrup prepared from waste bread

Bread type	Hardness day 1	Hardness day 4	Specific volume
Control wheat bread			
C-0	264^{e}	461^{f}	3.6^{a}
C-2	184^{abc}	322^{abc}	4.1^{fg}
C-4	178^{ab}	331^{bc}	4.2^{gh}
Bread with untreated slurry			
BSL-2	240^{de}	410^{def}	3.7^{abc}
Breads with hydrolyzed slurry			
BHSL-ALPHA-AMYL-2	196^{bc}	328^{bc}	4.0^{def}
BHSL-ALPHA-AMYL-4	186^{abc}	363^{cd}	4.0^{def}
BHSL-ALPHA-MALT-2	202^{bcd}	362^{cd}	3.8^{bcd}
BHSL-ALPHA-MALT-4	253^{e}	435^{ef}	3.7^{abc}
BHSL-ALPHA-AMYL-PROT-2	169^{ab}	291^{ab}	4.2^{gh}
Breads with syrup			
BSY-ALPHA-AMYL-2	186^{abc}	321^{abc}	4.1^{fg}
BSY-ALPHA-AMYL-4	197^{bc}	375^{cde}	3.9^{cde}
BSY-ALPHA-MALT-2	178^{ab}	297^{ab}	4.0^{ef}
BSY-ALPHA-MALT-4	224^{cde}	405^{def}	3.7^{abc}
BSY-ALPHA-AMYL-PROT-2	147^{a}	264^{a}	4.3^{h}

Note: Slurries and syrups were added at concentrations necessary to reach 2 or 4% of glucose or maltose in the bread dough (based on their glucose or maltose concentration). Control breads are prepared as regular wheat bread with or without sucrose addition. Bread type coding: C = control wheat bread; BSL = bread with untreated waste bread slurry (not hydrolyzed); BHSL = bread with added hydrolyzed slurry; BSY = bread with added syrup; ALPHA-AMYL, α-amylase and amyloglucosidase (0.05 and 2.5 g/kg bread, respectively); ALPHA-MALT, α-amylase and maltogenic amylase (0.05 and 2.5 g/kg bread, respectively); ALPHA-AMYL-PROT, α-amylase, amyloglucosidase, and protease (0.05, 2.5, and 4.6 g/kg bread, respectively); the number 0, 2, or 4 indicates the sugar level per flour weight. Different superscripts after each number indicates significant difference ($p < .05$ one-way ANOVA) between values within each column.

of waste bread treated without enzymes (BSL-2) had similar hardness to the control bread baked without sucrose (Table 3). All the breads baked with enzymatic hydrolyzed slurries or syrups with 2% glucose/maltose had a similar hardness compared to the wheat control bread (with 2% sucrose added) (Table 3). Comparing the breads containing corresponding hydrolyzed slurries and syrups, no difference in hardness was observed at 2% sucrose replacement. The bread made with ALPHA-AMYL-PROT syrup was the softest. These observations were valid for the hardness measured on Day 1 and Day 4. When the level of sucrose replacement was increased to 4%, breads enriched with ALPHA-MALT hydrolyzed slurry (BHSL-ALPHA-MALT-4) or syrup (BSL-ALPHA-MALT-4) were significantly harder than the corresponding breads at 2% sucrose replacement (BHSL-ALPHA-MALT-2 and BSL-ALPHA-MALT-2) and also harder than the breads enriched with glucose-rich slurry (BHSL-ALPHA-AMYL-4) and syrup (BSL-ALPHA-AMYL-4). The springiness and resilience results of all test breads showed no major difference between bread types in both Day 1 and Day 4 measurements.

All the breads baked with slurries or syrups enzymatic hydrolyzed with AMYL-ALPHA had higher volume than bread baked with the untreated waste bread (BSL-2). At 2% sucrose level, breads baked with ALPHA-AMYL had similar volume to the control wheat bread (4.1 mL/g). The bread BSY-ALPHA-AMYL-PROT-2 had the highest volume. For the breads with hydrolyzed slurry, the ones prepared with ALPHA-AMYL had higher volume than the ones prepared with ALPHA-MALT. However, no difference was observed in the volume of breads prepared with the syrups rich in glucose (BSY-ALPHA-AMYL-2) or maltose (BSY- ALPHA-MALT-2). When the level of sucrose was 4%, all the breads baked with enzymatic hydrolyzed slurries or syrups had smaller volume than the control wheat bread (C-4) (Table 3).

3.3 | Techno-economic assessment

An industrial-scale bakery waste recycling concept based on waste bread hydrolysis and recycling of the hydrolyzed slurry to bread dough preparation was designed, simulated, and compared with the current mean of bakery to dispose its waste to bioethanol production. The mass and energy balances for the Reference and Recycling concepts were calculated and used for evaluating the variable and fixed costs. Table 4 depicts the total annual production cost (€/a), the unit production costs (€/t bread), and the cost breakdown (% of total cost) for both evaluated concepts. The annual wheat bread, waste wheat bread, and waste raw dough production rates were 1065 t, 93 t, and 23 t, respectively. The total raw material costs in the Reference concept were 402 €/t bread. Compared to the Reference concept, the substitution of sugar (dosed as syrup) in the dough with the glucose of the hydrolyzed slurry decreased the total raw material costs by 11% to 358 €/t bread when all of the slurry was recycled back to dough preparation. By recycling all of the slurry, a sugar content of 4.8% f.w. in dough was achieved. The savings followed also from the substitution of the flour with the non-sugar dry matter of the slurry, and from the decreased yeast dosage. In the Recycling concept, there was also an 80% saving in the fee deriving from the disposal of the bakery waste to bioethanol production. The total FCI for the Recycling concept was 5.8 k€. Capital charges were 0.7 €/t bread and other fixed costs were 0.02 €/t bread. The total production costs for the Reference concept were 454 €/t bread and for the Recycling concept were 401 €/t bread. Compared to the current mean of disposing the bakery waste to bioethanol production, hydrolyzing the waste wheat bread and utilizing the whole hydrolyzed slurry in dough preparation is 12% more economical. The most crucial cost contributors were wheat flour (52–57% of total production costs), yeast (23–34%), and syrup (0–6%). Sensitivity analysis was carried out on the major variables affecting the profitability of the process (Figure 4). Variation in the wheat flour and syrup unit prices had a considerable impact on the unit production cost. If the wheat flour price decreases or the syrup unit prices increases

TABLE 4 The total annual production cost (€/a), the unit production cost (€/t bread), and the cost breakdown (% of total cost) for the current mean (Reference) and new mean to treat bakery waste (Recycling)

Reference concept	Component	Quantity	Unit cost	Cost (€/a)	Cost (€/t bread)	Share of total cost (%)
Raw materials	Emulgator	10.0 t/a	3000 €/t	30,000	28.2	6.2
	Enzyme	0 t/a	7500 €/t	0	0	0
	Preservative	0.76 t/a	4000 €/t	3050	2.9	0.6
	Salt	11.7 t/a	45 €/t	530	0.50	0.1
	Syrup	30.3 t/a	1000 €/t	30,300	28.5	6.3
	Wheat flour	1000 t/a	250 €/t	250,000	235	51.8
	Yeast	50 t/a	2250 €/t	112,500	106	23.3
	Water	603 t/a	3 €/m^3	1810	1.7	0.4
Utilities	Natural gas	942 MWh/a	42 €/MWh	39,560	37.2	8.2
	Electricity	50 MWh/a	71 €/MWh	3520	3.3	0.7
Wastes	Waste to bio-EtOH	116 t/a	100 €/t	11,620	10.9	2.4
Other costs	Labor	0	0	0	0	0
	Maintenance	0	1.5% of FCI	0	0	0
	Insurances, taxes	0	1.5% of FCI	0	0	0
Capital charges	Annualized FCI	0	10 years, 5%	0	0	0
Total				482,890	454	100
Recycling concept	**Component**	**Quantity**	**Unit cost**	**Cost (€/a)**	**Cost (€/t bread)**	**Share of total cost (%)**
Raw materials	Emulgator	10.0 t/a	3000 €/t	30,000	28.2	7.0
	Enzyme	0.18 t/a	7500 €/t	1320	1.24	0.3
	Preservative	0.76 t/a	4000 €/t	3050	2.9	0.7
	Salt	10.8 t/a	45 €/t	480	0.45	0.1
	Syrup	0 t/a	1000 €/t	0	0	0
	Wheat flour	973 t/a	250 €/t	243,370	229	57.0
	Yeast	45 t/a	2250 €/t	101,250	95	23.7
	Water	585 t/a	3 €/m^3	1760	1.65	0.4
Utilities	Natural gas	940 MWh/a	42 €/MWh	39,470	37.1	9.2
	Electricity	49 MWh/a	71 €/MWh	3510	3.3	0.8
Wastes	Waste to bio-EtOH	23 t/a	100 €/t	2320	2.2	0.5
Other costs	Labor	0	0	0	0	0
	Maintenance	—	1.5% of FCI	10	0.01	0.003
	Insurances, taxes	—	1.5% of FCI	10	0.01	0.003
Capital charges	Annualized FCI	—	10 years, 5%	750	0.7	0.2
Total				427,300	401	100

by 50%, the Recycling concept is 14% more economical than the Reference concept.

4 | DISCUSSION

In this work, waste bread was efficiently hydrolyzed by amylolytic enzymes (and proteases) to produce mono- or disaccharides and subsequently successfully used either as a bread slurry or as a syrup (after solid–liquid separation) in wheat bread making. The best dosage of enzymes, that is combination of α-amylase (0.05 g/kg bread) and amyloglucosidase (2.5 g/kg bread), resulted in a syrup containing 22% of glucose. When comparing to the previous data, only few works were found in the literature related to waste bread hydrolysis. Hudečková et al. (2017) and Benabda et al. (2018) reported the highest glucose concentration as 70 and 60.6 g/L, respectively, for waste bread hydrolyzed with α-amylase and glucoamylase. The lower value reported by these authors was probably due to the more dilute

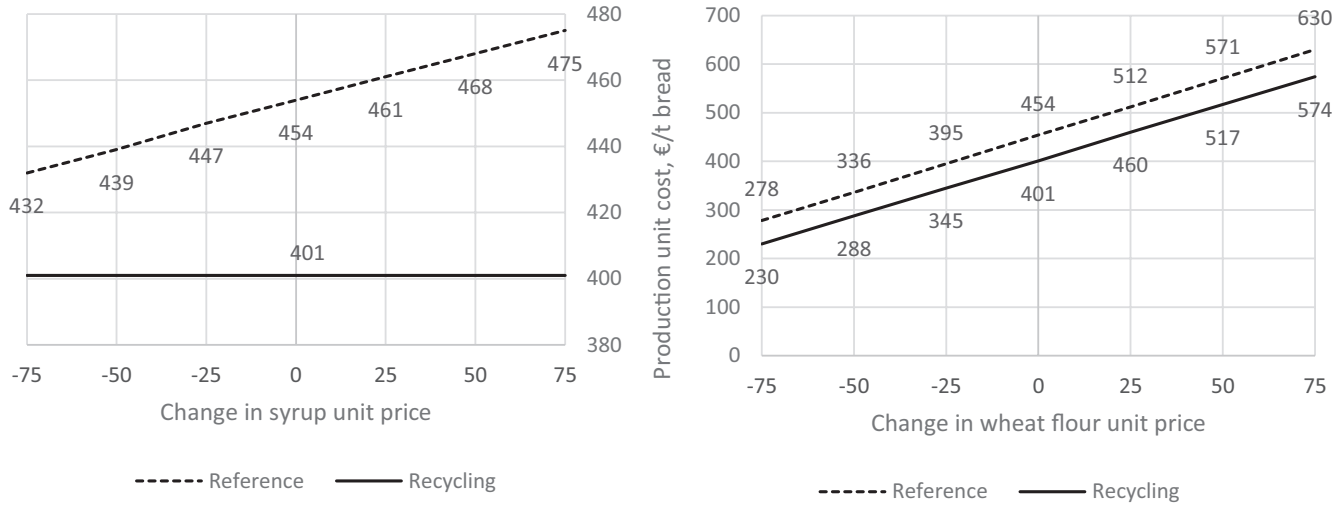

FIGURE 4 Sensitivity analysis of the unit production cost (€/t bread) on key economic parameters for the current mean (Reference) and the new mean to treat bakery waste (Recycling)

systems and shorter time when compared to the present work. Sükrü Demirci et al. (2017) used a response surface methodology to optimize the enzymatic hydrolysis of waste bread and reported that 99% of theoretical maximum glucose yield was achieved, which was very close to the 93% glucose yield found in the present work. The best hydrolysis condition reported by Sükrü Demirci et al. (2017) was with a slurry concentration 0.05 wt/wt (waste bread/water), 0.03 KNU/g substrate of a-amylase (pH 6, 90°C, 60 min), and 3.6 AGU/g substrate of glucoamylase (pH 5, 55°C, 24 hr). When comparing to the present work, it is important to point out that the previous reports (Hudečková et al., 2017; Sükrü Demirci et al., 2017) have used a thermostable α-amylase for the liquefaction of waste bread at high temperatures (80–90°C), which differs from the present work where α-amylase at 50°C was used simultaneously with exoamylases. However, the liquefaction in the present work at 50°C seemed to work as well as at high temperatures reported (80–90°C). This is most probably because starch in bread was already gelatinized and prone to be easily degraded by amylases. Moreover, the conditions used in this work were chosen in order to be easily performed in the industry, as for example, avoiding drying/grinding process of waste bread, using slurry with high-solid content and a steady process (no special mixing required).

The use of maltogenic amylase to produce waste bread-based ingredients rich in maltose was also evaluated. Maltogenic amylase combined with α-amylase was much less efficient than amyloglucosidase, and a syrup containing 12% of maltose was produced. Gaouar et al. (1998) reported that a massive dosage of maltogenic amylase was required to achieve high maltose concentrations when converting cassava starch into maltose by using an ultrafiltration membrane reactor. In the present work, no clear dose–response was observed for maltogenic amylase. The formation of maltose probably has inhibited the action of α-amylase as maltose was shown to be a competitive inhibitor of amylase action (Warren et al., 2012). Amyloglucosidase had a dose-dependent effect, which was more important than the concentration of α-amylase. These results are in agreement with Sükrü Demirci et al. (2017), who reported a higher glucose yield upon glucoamylase increase but no dose-effect by α-amylase in the enzymatic hydrolysis of waste bread. In the present study, it seems that the dosage of amyloglucosidase could be even further reduced, reducing therefore the process costs.

Enzymatically hydrolyzed waste bread samples (i.e., hydrolyzed bread slurries and syrups obtained by centrifugation of the hydrolyzed slurry) were used to replace sucrose and water in bread baking. The 2% sucrose replacement was chosen as a typical level in bread baking and the level was also increased to 4% to evaluate the impact of the surplus of the waste bread slurry and syrup samples on bread quality. First, the positive impact of using enzymes in waste bread liquefying was observed as the breads baked with control slurry (i.e., waste bread treated only with water and without enzymes) were harder and smaller to breads made with hydrolyzed waste bread (slurry or syrups). Second, all the breads baked with enzymatically hydrolyzed slurries or syrups maintained the quality of wheat-baked breads compared to the control bread with 2% sucrose added. This indicated successful sugar replacement by the waste bread originated samples. Previous reports on recycling waste bead into new bakery products are rare. Gélinas et al. (1999) have used waste bread to produce sourdough, but the impact of the sourdough on bread baking was not evaluated.

The addition of hydrolyzed bread slurry or syrup from waste bread at 2% glucose/maltose enrichment level did not affect the final characteristics of breads when compared to the control bread using 2% sucrose. Glucose and fructose-rich syrups can efficiently replace sucrose in bakery products (Zargaraan et al., 2016). Still, it was interesting to observe that the use of hydrolyzed slurry did not cause deterioration on final bread quality. Another positive effect in bread baking was observed with the waste bread samples

produced with proteases combined with amylolytic enzymes. The higher amount of soluble protein in the system made breads softer and with higher volume. These solubilized proteins with small molecular weight have been probably incorporated into the aqueous phase and created air bubbles more efficiently by lowering the surface tension (Wilde, 2012). Moreover, the denaturated insoluble gluten could cause physical hindrance for the new gluten network and its removal by proteases might have contribute to the dough structure formation. Concerning the differences between samples rich in glucose or maltose, it was observed that breads baked with hydrolyzed slurry rich in maltose were harder and smaller than the bread baked with glucose-rich samples. The detrimental effect of the hydrolyzed slurry rich in maltose could be due to the slower use of maltose as main substrate by baker's yeast, as observed by the longer proofing time needed.

5 | CONCLUSIONS

This study showed that enzyme-aided bread hydrolysis is a potential approach to recycle the waste bread back to the wheat bread-making process. Both hydrolyzed waste bread slurry and syrup prepared by solid–liquid separation from the slurry replaced sucrose in bread without affecting the quality of the final product, that is new breads produced with waste bread hydrolysis products had similar texture and volume to the control bread using sucrose. Breads prepared with hydrolyzed slurries rich in glucose had better properties than the ones containing maltose, but no difference was observed between the glucose- and maltose-rich syrups. The addition of protease during waste bread hydrolysis was shown to slightly improve the volume and softness of the new breads. The food-grade enzymes used in this work are already commonly used in bakery applications. Amylases are usually used during bread making anti-staling agents. Therefore, their use in bakeries to reprocess waste bread in a circular process seems feasible, reasonable, and sustainable. A conceptual-level TEA was conducted to evaluate the economic feasibility of the novel approach to treat the waste wheat bread inside the bakery by hydrolyzing it and recycling the hydrolyzed slurry back to bread making. The TEA revealed that the new approach is 12% more economical than the current mean to dispose the bakery waste to bioethanol production. When using this recycling approach, safety and quality control protocols would be required to mitigate any kind of microbiological risk in the new baked products.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Natalia Rosa-Sibakov: Conceptualization; data curation; formal analysis; investigation; methodology; resources; validation; writing – review and editing. **Lotta Sorsamäki:** Conceptualization; data curation; formal analysis; investigation; methodology; writing – review and editing. **Mikko Immonen:** Formal analysis; investigation; methodology. **Hanna Nihtilä:** Investigation. **Henry Ndegwa Maina:** Conceptualization; funding acquisition; supervision. **Matti Siika-Aho:** Conceptualization; funding acquisition; project administration; resources; writing – review and editing. **Kati Katina:** Conceptualization; funding acquisition; methodology; supervision. **Emilia Nordlund:** Conceptualization; funding acquisition; project administration; resources; writing – review and editing.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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