Valorization of industrial bread waste using enzymatic treatment and sourdough fermentation

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This PhD project aims to develop a protocol for recycling of bread waste through a combination of enzymatic hydrolysis and sustainable, low-cost and green biotechnology of fermentation, recently rediscovered as a feasible alternative to enhance the technological, nutritional, sensory and functional features of agro-food by-products. “Bread slurries”, obtained by mixing bread waste flour and water, were enzymatically hydrolyzed by proteolytic and amylolytic enzymes, alone and in combination followed by fermentation with *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae* as starters.

Valorizzazione degli scarti di pane mediante trattamento enzimatico e fermentazione con lievito madre

Questo progetto di ricerca è finalizzato al recupero degli scarti di pane attraverso la combinazione dell'idrolisi enzimatica e della biotecnologia low-cost, green e sostenibile della fermentazione, recentemente riscoperta come alternativa per recuperare e/o migliorare le caratteristiche tecnologiche, nutrizionali, sensoriali e funzionali dei sottoprodotti agroalimentari. Gli "impasti di pane", ottenuti mescolando farina di scarto di pane e acqua, sono stati idrolizzati con enzimi proteolitici e amilolitici, da soli e in combinazione, seguiti da fermentazione con *Lactiplantibacillus* *plantarum* e *Saccharomyces* *cerevisiae* come starter.

**Key words**: bread waste, enzymatic hydrolysis, sourdough fermentation, sustainability

# **1. Introduction**

Nowadays, one of the most important categories of food waste is represented by leavened baked goods, such as bread. Over the last decade, many researchers have attempted to find recycling alternatives (Verni et al., 2020). According to the PhD thesis project previously described, this poster reports the main results obtained from the enzymatic hydrolysis and fermentation of the “bread slurries”. The optimal amount of water and bread waste flour, type of the enzymes (alone or in combination) and their concentrations, type of starters and time - temperature needed for the enzymatic treatment and fermentation, were determined as the main process parameters.

# **2. Materials and Methods**

White wheat bread crusts were ground and mixed with an optimal distilled water ratio, as previously described (Verni et al., 2021), and homogenized with a blender to obtain “bread slurries”, that were subjected to enzymatic hydrolysis and fermentation for 24 h at 30°C. Four enzymes, alone and in combination, were used to hydrolyze bread slurries: glucoamylase (AMYL), amylase fongique (FONG), protease (PROT) and amylase-protease mix (MIX). *Lactiplantibacillus plantarum* and *Saccharomyces cerevisiae* were used as starters for the fermentation. The enzymatically hydrolysed and fermented bread slurries were evaluated for microbial growth (Log CFU/mL) and acidification kinetics, total titratable acidity (TTA), as well as sugars (maltose, glucose, and fructose), organic acids (lactic and acetic acids), proteins, peptides and free amino acids (FAA) concentrations. Consequently, the best performing sourdough in terms of microbial cell density, acidification and lactic acid production was subjected to bread making trials. Different percentages of the prepared sourdough, i.e., 10%, 20%, 30% and 50% were used for breadmaking. All the breads were characterized for pH, TTA, leavening capacity, texture, specific volume, alveolation, and color.

# **3. Results and Discussion**

To select the “enzyme + microorganism” combination that shows the fastest acidification at the end of 24 h fermentation, the growth kinetics of **lactic acid bacteria and yeasts** in the individual bread slurries, with or without enzymatic hydrolysis, was determined using the Gompertz equation, as modified by (Zwietering et al., 1990)

 (1)

where A is the cell density variation (between inoculation and stationary phase); µmax is the maximum growth rate expressed as units/h and λ is the length of the lag phase measured in hours. **As reported and highlighted by the circle in Figure 1, bread slurries fermented with *L. plantarum* and *S. cerevisiae* and enzymatically hydrolyzed with protease and amylase-protease mix showed the highest cell densities for both lactic acid bacteria and yeasts** t**han the corresponding fermented slurries without the enzymatic treatment.**

Enzymes + *L. plantarum*

Enzymes + *S. cerevisiae*



Log CFU/mL

Log CFU/mL



Time (h)

Time (h)

**Figure 1.** *Growth kinetics of lactic acid bacteria and yeasts alone and in combination with the four different enzymes*

The highest cell growth (Log CFU/mL) of lactic acid bacteria and yeasts in these two samples was also confirmed by the one-way ANOVA with Post-hoc Tukey’s comparison (P < 0.05). Accordingly, a faster acidification in the “protease + *L. plantarum*” and in the “mix amylase-protease + *L. plantarum*” samples was observed, in accordance with a more than twofold increase in lactic acid concentration compared to the other treated bread slurries.

Therefore, the protease enzyme was selected for the preparation of two different sourdoughs, with the aim of evaluating the effect of combining lactic acid bacteria and yeast: protease + *L. plantarum* (7 Log CFU/mL) and protease + *L. plantarum* (7 Log CFU/mL) + *S. cerevisiae* (5 Log CFU/mL). The quantification of sugars in the sourdough by HPLC analysis revealed that the presence of *S. cerevisiae* caused the depletion of glucose, fructose, and maltose after 24 h fermentation, in agreement with the literature (Paramithiotis et al., 2006), whereas in the “protease + *L. plantarum*” sourdough (where *S. cerevisiae* is not present), maltose was not metabolized.

Finally, the two sourdoughs from bread waste were used in different percentages for bread making trials. The evaluation of the leavening capacity and acidification of the final bread doughs demonstrated the differences among bread doughs prepared using different sourdoughs as well as among bread doughs prepared using the same sourdough but different inoculation percentages of the latter.

**It was observed that the specific volume of the final bread decreased with the increasing percentage of sourdough. More specifically, when 10% and 50% sourdough (prepared with protease + *L. plantarum)* was used, the specific volume of the final bread was 2.53 ± 0.02 mL/g and 1.64 ± 0.04 mL/g, respectively, whereas when 10% and 50% of sourdough (prepared with protease + *L. plantarum* + *S. cerevisiae)* was used, the specific volume of the final bread was 2.23 ± 0.16 mL/g and 1.87 ± 0.02 mL/g, respectively.**

Therefore, in combination with texture and sensory analyses of these breads, our results indicated the application of enzymatically treated sourdough as a baking ingredient, thus enabling the recycling of bread waste in a sustainable and low-cost circular economy concept.

# **4. Future perspectives**

All the chemical-physical-sensorial parameters of the bread will be considered to define the best fermentation conditions and inoculum percentage. For the next activities, the enzyme concentration for the enzymatic treatment, the protease activity of the bread waste sourdough and the type of starter cultures for sourdough fermentation will be taken into account with a view to further optimise the process.

# **5. References**

Paramithiotis, S., Gioulatos, S., Tsakalidou, E. and Kalantzopoulos, G., 2006. Interactions between Saccharomyces cerevisiae and lactic acid bacteria in sourdough. *Process Biochemistry*, *41*(12), pp.2429-2433.

Verni, M., Vekka, A., Immonen, M., Katina, K., Rizzello, C.G. and Coda, R., 2022. Biosynthesis of γ‐aminobutyric acid by lactic acid bacteria in surplus bread and its use in bread making. *Journal of Applied Microbiology*, *133*(1), pp.76-90.

Verni, M., Minisci, A., Convertino, S., Nionelli, L. and Rizzello, C.G., 2020. Wasted bread as substrate for the cultivation of starters for the food industry. *Frontiers in Microbiology*, *11*, p.293.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M. and Van't Riet, K.J.A.E.M., 1990. Modeling of the bacterial growth curve. *Applied and environmental microbiology*, *56*(6), pp.1875-1881.