Effect of fermentation of selected lactic acid bacteria on the technological properties of sorghum-composite bread

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The first 2 activities of the Ph.D. project are described. The growth capacity of selected lactic acid bacteria on sorghum and their effect on the thermal, rheological, molecular, antioxidant, and color properties of sorghum flour were evaluated. The effect of fermentation on the technological properties of sorghum-composite bread was then evaluated in terms of specific volume, texture, *a*w, moisture content, color, and sensory acceptability.

Effetto della fermentazione da parte di batteri lattici selezionati sulle proprietà tecnologiche di pane composito di sorgo

Vengono descritte le prime 2 attività del progetto di tesi di dottorato. È stata misurata la capacità di crescita di batteri lattici selezionati sulla farina di sorgo e il loro effetto sulle proprietà termiche, reologiche, di mobilità molecolare, di colore e di capacità antiossidante, sulla farina di sorgo. È stato poi valutato l’effetto dal punto di vista tecnologico della fermentazione della farina di sorgo sul pane composito, valutando il volume specifico, la consistenza, *a*w, il contenuto di acqua, il colore, e l’accettabilità sensoriale.

**Keywords**: sorghum, bread, functional properties, LAB fermentation.

# **1. Introduction**

Following the previously described Ph.D. project (Chiodetti, 2022), this poster reports the main results of the first two activities concerning:

(A1) Selection of functional LAB strains and subsequent fermentation of sorghum flour. Determination of the effect of the LAB activity on the techno-functional properties sorghum flour;

(A2) Evaluation of the effect of the addition of sorghum liquid sourdough on the properties of fresh wheat composite bread. Assessment of bread consumer acceptability.

# **2. Materials and Methods**

*Lactobacillus delbruekii* subsp. *bulgaricus* 1932, *Lacticaseibacillus casei* 4339, and *Leuconostoc* spp. 4454, previously selected according to their proteolytic, aromatic, and EPS-producing activities, were used for the liquid sourdough fermentation of sorghum flour (25°C, 15h). To assess the growth capacity of bacteria on sorghum, the pH, total titratable acidity (TTA), and microbial counts in the sourdoughs were analyzed. Moreover, the color, antioxidant activity (DPPH assay), viscosity (rheometer; 25°C, 0.1-1000 1/s), pasting properties (rheometer; 160 rpm, heating 50-95°C, holding 95°C for 5 min, cooling 95-25°C, 5°C/min), thermal properties (DSC; heating 30-130°C, 5 °C/min), and 1H molecular mobility (Time Domain 1H NMR; self-diffusion coefficient D, CPMG 1HT2) were evaluated in the sourdough samples. To evaluate the effect of fermentation on sorghum-composite bread, wheat bread with 25% sorghum sourdough and a control bread (from wheat and unfermented sorghum flour), were characterized in terms of specific volume, *aw,* moisture content, color, texture (TPA, 40% compression, 35 mm cylindrical probe) and antioxidant activity. The consumer sensory acceptability of sorghum-composite breads was evaluated with an acceptability test (n = 58 untrained judges), using a nine-point hedonic scale. Overall, three independent batches were analyzed for each sample. Statistical differences were evaluated with one-way ANOVA and Tukey post hoc test (α=0.05) with SPSS software (v. 27.0, SPSS Inc., Chicago, USA).

# **3. Results and Discussion**

## **3.1 Effect of fermentation on the functional properties of sorghum flour**

All LAB strains showed excellent growth capacity on sorghum, reaching values of up to 109 CFU g-1, and pH between ≈ 4.2 and 4.5 (Table 1). Moreover, fermentation increased the total titratable acidity of sourdoughs. After fermentation, the increase in the acidity and consequently a decrease in pH of sourdoughs showed a pH-induced color change, with increased a\*, b\*, and L\* in fermented samples. The changes that occurred in the color properties can be probably related to changes affecting the phenolic compounds (Olojede et al., 2022). The antioxidant activity also increased after fermentation (with the highest value for sample *L*. 4454). This result can be related to the effect of hydrolysis and the release of bound antioxidant compounds during fermentation and *de novo* synthesis of compounds with antioxidant activity (Gobbetti et al., 2019). Furthermore, fermentation increased the viscosity of sourdoughs, especially in the sample *Lcb.* 4339, which showed the highest viscosity. The increase in viscosity could be due to a hypothetical effect of EPS production and changes in the proteins and starch fractions.

In all samples, fermentation increased the ability of starch to gelatinize, as an increase in the enthalpy of the endothermic peak of starch was detected in DSC. Fermentation by *Lb*. 1932 and *Lcb*. 4339 also increased peak and final viscosity of corresponding sourdoughs. However, the fermented samples showed an increase of the onset temperature of the starch gelatinization and, in *Lcb*. 4339, an increase in the pasting temperatures. The increase in gelatinization temperatures may be related to structural changes in the amylopectin fraction occurring during fermentation (Ye et al., 2019).

The 1H NMR analysis suggested that the highest matrix breakdown occurred in *Lb*. 1932, as highlighted by the highest value of the self-diffusion coefficient 1H D in this sample. The matrix breakdown was also suggested by the higher number of 1H T2 populations in the fermented samples (four populations: A, B, C, D) compared to the standard (three populations). Among the fermented samples, *Lb.*1932 showed the highest abundance and relaxation time (ms) of Pop D; this population represents the weakly bound OH protons of water. In contrast, the highest abundance of Pop C, related to the exchanging protons of water interacting with starch and proteins, was found in *Lcb*. 4339 (Marchini et al., 2021).

***Table 1*** *pH**of sourdough (SD), bread dough (D), and bread (B) of the experimental samples. STD = unfermented; Lb. 1932 = fermented with Lactobacillus delbruekii subsp. bulgaricus 1932; Lcb 4339 = fermented with Lacticaseibacillus casei 4339; L. 4454 = fermented with Leuconostoc spp. 4454. Different letters indicate significant differences (p≤0.05) between different samples for the same substrate.*

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| --- | --- | --- | --- | --- |
| ***pH substrate*** | ***STD*** | ***Lb. 1932*** | ***Lcb. 4339*** | ***L. 4454*** |
| ***SD*** | *6.26±0.01 a* | *4.48±0.04 b* | *4.24±0.02 c* | *4.36±0.09 bc* |
| ***D*** | *5.54±0.02 a* | *5.15±0.03 b* | *5.03±0.01 c* | *5.05±0.01 c* |
| ***B*** | *5.47±<0.01 a* | *5.07±0.02 b* | *4.96±0.04 c* | *5.02±0.01 bc* |

## **3.2 Effect of sorghum fermentation on the bread overall quality**

The specific volume of the bread loaves did not change after fermentation, except for sample *Lcb*.4339, in which specific volume resulted slightly but significantly reduced, probably due to the acidic weakening of the gluten network and thus the ability to retain gas during rising and baking (Su et al., 2019) (Figure 1). The fermented loaves also had a higher crumb moisture content. The hypothetical presence of water-binding molecules, such as EPS, could explain the higher crumb moisture in fermented bread, as they could have reduced water loss during baking (Lynch et al., 2018). The color changes for the a\* parameter found in the sourdough were also evident in the crumb, which showed a more intense red color compared to the standard. The texture properties improved after fermentation in *Lb*.1932 and *L*. 4454 loaves, showing higher cohesiveness. On the other hand, sample *Lcb* 4339 was harder than the standard. However, regarding the bread sensory acceptability test, there were no significant differences on the texture and color of the loaves, while fermentation improved the overall appearance in sample *Lcb*. 4339. Finally, sample *Lb*. 1932 had the least pleasant odor acceptability. Therefore, these results showed that fermentation can improve some properties of sorghum composite bread. However, more in-depth studies are needed to verify the production of EPS, the role of fermentation in bread staling, and the effect of different methods of addition of sourdough on bread quality, following the original thesis project.

***Figure 1*** *Experimental bread samples. STD = control bread; Lb. 1932 = bread fermented with Lactobacillus delbruekii subsp. bulgaricus 1932; Lcb 4339 =. bread fermented with Lacticaseibacillus casei 4339; L. 4454 = bread fermented with Leuconostoc spp. 4454.*

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