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Different strategies to enhance mould-free bread shelf life

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Introduction

Mould contamination is one of the main issues for bread producers. Conidiospores of filamentous fungi are dispersed by air, and control of contamination requires clean room technology. The water activity and the pH of bread support germination and growth of fungal spores. Microbial fermentation is one of the oldest and ecologically friendly methods of preserving foods. LAB produce compounds with demonstrated antifungal activity (Black et al., 2013; Quattrini et al., 2018). The use of may change the mould-free shelf life of bread. Flaxseeds are rich source of omega-3 fatty acids offers numerous health benefits, particularly related to prevention of cardiovascular diseases. The high linoleic acid content can also enhance the antifungal activity of sourdough. Black et al. (2013) demonstrated that lactobacilli convert linoleic acid to antifungal hydroxyl fatty acids. The products, 10-hydroxy-octadecenoic acid, and its isomers, coriolic acid and ricinoleic acid, have similar antifungal activities (Liang et al., 2017) and can be valuable in delaying mould growth in bread. This work aimed to identify alternative natural strategies to chemical preservatives in order to prevent or delay fungal spoilage of bread. We compared *in vitro* MIC –a checkerboard assay- of common bread preservatives (propionate and sorbate), acetic acid, 3-phenyllactic acid and ricinoleic acid with the *in situ* antifungal potential –challenge test. We also explored the use of flaxseed flour, which is of great nutritional value and could represent a valid functional alternative to wheat flour for sourdough fermentation.

Results

1) Minimum inhibitory activity of acetate in combination with other antifungal agents

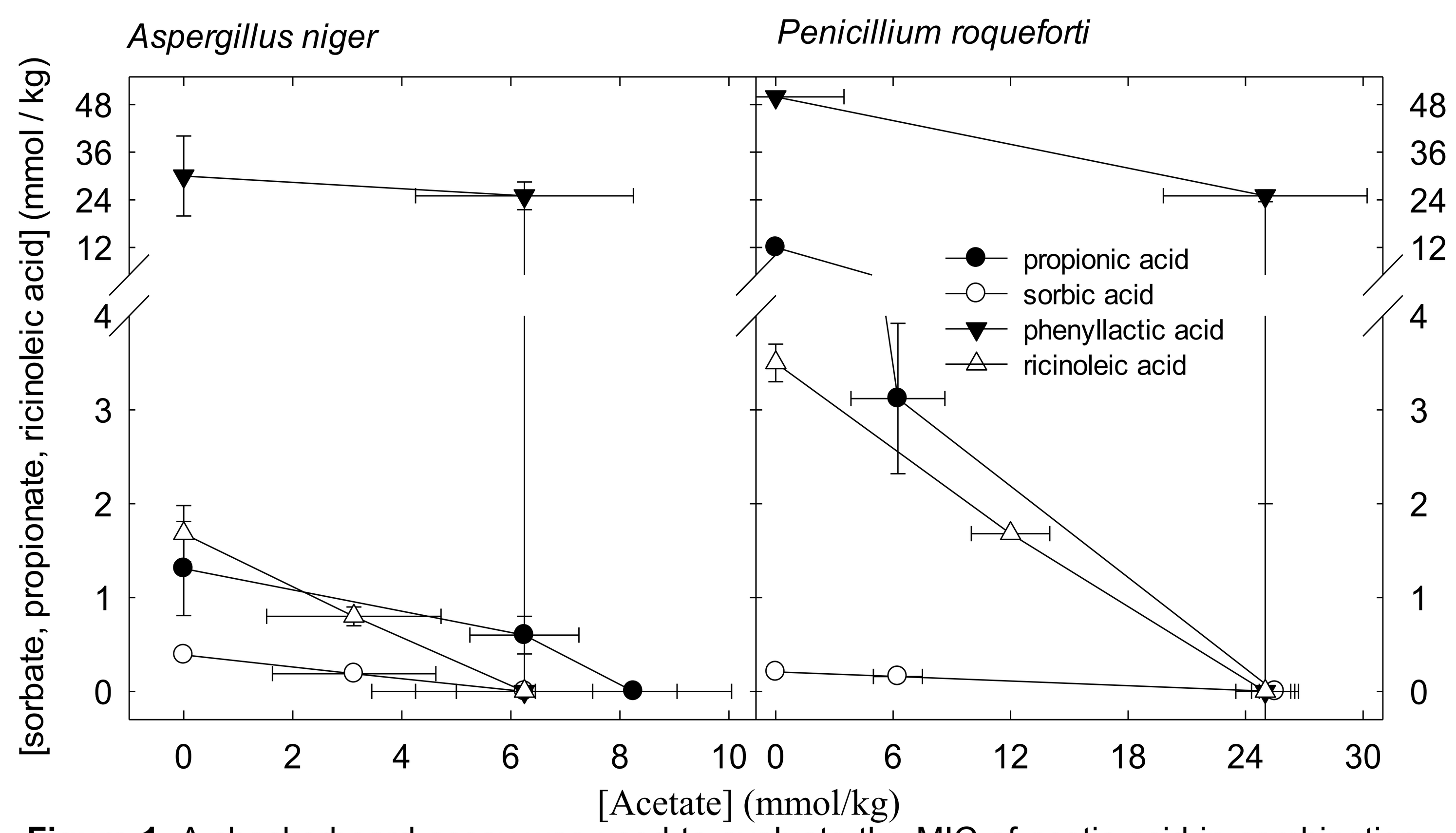


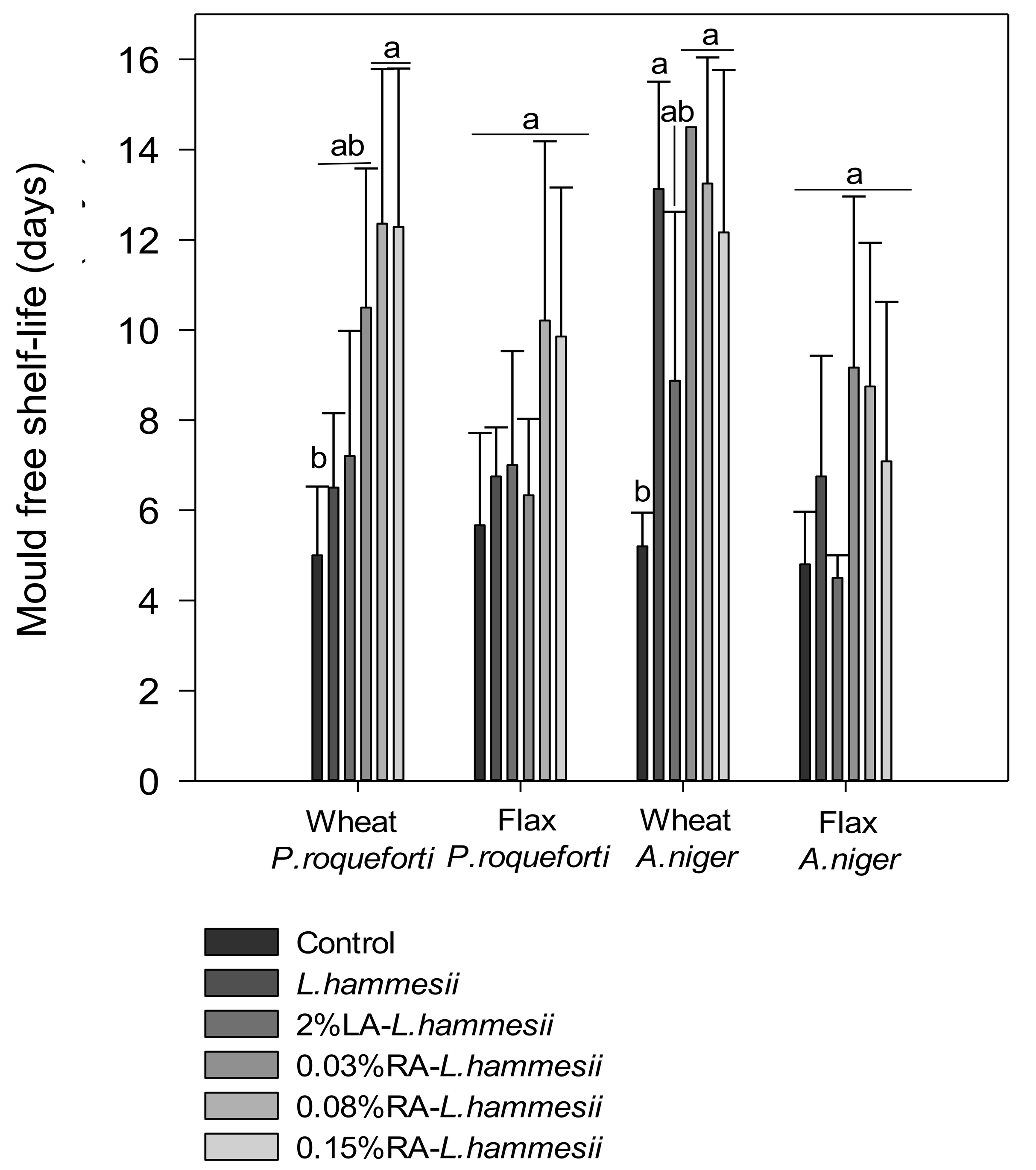
Figure 1. A checkerboard assay was used to evaluate the MIC of acetic acid in combination with sorbic acid, propionic acid, phenyllactic acid, or ricinoleaic acid. The MIC were evaluated at pH 4.5.

2) *In situ* inhibitory activity of antifungal agents

Indicator strains	Control	3-phenyllactic acid	Ricinoleic acid	Acetic acid	Calcium propionate	Sorbic acid	Calcium propionate + acetic acid	Sorbic acid + acetic acid
A. niger FUA5001	**3.6**±1.1^{a}	**5.3**±0.5^{a}	**4.3**±1.1^{a}	**9.7**±0.5^{b}	**8.3**±1.1^{b}	**10.0**±1^{b}	**8.5**±0.7^{b}	**6.0**±0.0^{ab}
P. roqueforti FUA5005	**4.3**±0.1^{a}	**5.0**±1.0^{a}	**4.7**±1.1^{a}	**9.3**±0.5^{b}	**8.0**±1.0^{b}	**9.0**±0.7^{b}	**7.5**±0.3^{ab}	**6.5**±0.7^{ab}

In situ effect of chemical preservatives and their combination on bread mould-free shelf life (expressed in days). The chemicals were used at the level of their MIC **(Figure 1)** . Results of three independent experiments are shown as means ± standard deviations. Differences were considered at $p<0.05$.

4) *In situ* inhibitory activity of ricinoleic acid in combination with *L. hammesii* sourdough



In situ effect of wheat or flaxseed sourdough combined with hydroxy unsaturated fatty acids on bread mould-free shelf life (expressed in days). Bread experiment groups included control without addition of sourdough and ricinoleic acid; *L.hammesii*-fermented sourdough bread without addition of ricinoleic acid (*L. hammesii*); *L.hammesii*-fermented sourdough bread with addition 20 g/L linoleic acid (2%LA-*L.hammesii*); *L.hammesii*-fermented sourdough bread with addition of 0.03%, 0.08% and 0.15% ricinoleic acid (%RA-*L.hammesii*).

3) *In situ* inhibitory activity of antifungal agents in wheat and flaxseed bread in combination with *L. hammesii* sourdough

Indicator strains	Not fermented wheat	Not fermented flaxseed	*L. hammesii* wheat	*L. plantarum* wheat	*L. brevis* wheat	*L. hammesii* wheat + calcium propionate	*L. hammesii* wheat + sorbic acid	*L. hammesii* flaxseed	*L. plantarum* flaxseed	*L. brevis* flaxseed	*L. hammesii* wheat + sucrose	*L. plantarum* wheat + sucrose	*L. brevis* wheat + sucrose	*L. hammesii* flaxseed + sucrose	*L. plantarum* flaxseed + sucrose	*L. brevis* flaxseed + sucrose
A. niger FUA5001	**3.0**±0.6^{a}	**3.0**±0.0^{a}	**4.8**±0.3^{b}	**4.3**±0.6^{b}	**4.7**±0.6^{b}	**10.5**±0.7^{c}	**7.0**±1.4^{c}	**5.0**±0.6^{b}	**3.6**±0.6^{a}	**3.7**±0.6^{a}	**5.5**±0.7^{b}	**5.0**±0.0^{b}	**9.0**±0.0^{c}	**6.5**±0.0^{ab}	**5.0**±0.0^{b}	**9.0**±0.0^{c}
P. roqueforti FUA5005	**5.3**±0.6^{b}	**3.3**±0.6^{a}	**5.3**±0.6^{b}	**5.0**±0.0^{b}	**5.0**±0.0^{b}	**8.3**±0.3^{c}	**5.5**±0.7^{b}	**5.0**±0.0^{b}	**3.6**±0.6^{a}	**4.3**±0.6^{a}	**6.5**±0.7^{bc}	**5.5**±0.7^{b}	**8.5**±0.7^{c}	**6.5**±0.7^{b}	**5.5**±0.7^{b}	**8.5**±0.7^{c}

In situ effect of sourdough on bread mould-free shelf life (expressed in days). The sourdough was obtained with selected *L. hammesii, L. plantarum* or *L. brevis* strains and supplemented with lowered concentrations of calcium propionate (3.1 mM) or sorbic acid (0.16 mM). The addition of sucrose (4%) was also tested. The challenge test was conducted for 12 days against the two indicator strains. Data are the results of three independent experiments expressed as means ± standard deviations. The statistical difference was considered at $p<0.05$.

Conclusions

- *In vitro* MIC of calcium propionate, sorbic acid and acetic acid matched the *in situ* antifungal activity.
- Synergistic effect are demonstrated, lowering the concentrations of bread preservatives needed to delay the fungi development.
- Acetic acid is the most relevant antifungal metabolite of *Lactobacillus* spp. and exerts synergistic effect with other preservatives.
- *L. hammesii* sourdough with ricinoleic acid (0,08% or 0,15%) showed the longest mould free shelf life – 7 days more than the unfermented bread.
- The use of flaxseed flour didn't result in the extra protection on bread against moulds, indicating that bound linoleic acid was not converted to a sufficient quantity of free hydroxy fatty acids.

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