FACULTY OF
BIOSCIENCE ENGINEERING

LABORATORY OF APPLIED MYCOLOGY & LABORATORY OF CEREAL TECHNOLOGY, DEPARTMENT OF APPLIED BIOSCIENCES, FACULTY OF BIOSCIENCE ENGINEERING, GHENT UNIVERSITY, BELGIUM

Els Debonne, Filip Van Bockstaele, Simbarashe Samapundo, Frank Devlieghere, Mia Eeckhout

NATURAL ALTERNATIVE PRESERVATION STRATEGIES FOR BREAD PRODUCTS

Abstract

Microbiological spoilage of bread is primarily an issue for bread products intended to be stored at room temperature for a longer period than the time needed to be rejected because of staling, e.g. packaged sliced toast bread and par-baked bread products packed under modified atmosphere. Mould growth is the most important shelf-life limiting factor of bread products, with *Penicillium* spp. and *Aspergillus* spp. the most dominant species. MA-packaged par-baked bread can be stored for several weeks, though, due to high levels of water activity, these products are still highly sensitive to spoilage.

The aim of this study was to develop natural *clean label* preservation strategies for par-baked bread with attention for both **microbiological and technological bread quality**. The effectiveness of natural antifungals, including essential oils, sourdough and other natural fermentation products, was pre-screened with an optimized *in -vitro* assay based on micro-dilution of components whereby the assay permits to accurately model the minimal inhibitory concentration of oil or water soluble extracts. Further, the obtained information of *in-vitro* antifungal activity was compared to results of shelf-life and challenge tests of par-baked breads. Finally, the quality of fully baked par-baked bread with natural antifungals was assessed.

MA-packaged par-baked bread

The influence of the par-baking parameters, including par-baking temperature, time, steam, packaging and combinations thereof, is of particular interest as to provide the food industry recommendations concerning the optimal par-baking strategy for a better and longer preservation of par-baked breads without having to add a chemical preservative such as calcium propionate. The impact of par-baking on par-baked bread shelf-life is currently under investigation (including total anaerobic plate counts, spores of *Bacillus subtillis* and moulds and yeasts. However, in addition to the microbiological study, the impact of par-baking conditions on par-baked and fully baked bread was already investigated.

	Phase	Time (min)	Temperature (°C)	Steam volume (mL)	Steam valve
Par-baking	1	2	170/195/220	200/600	closed
	2	8/13	150/175/200	0	closed
Fully baking	1	1	220	400	closed
	2	8	200	0	closed
	3	2	210	0	open




Table 1 GLM of quality parameters of par-baked bread (y_i: volume (V), weight (W), specific volume ratio, crust thickness and L, a*, b*) in function of par-baking conditions (x_i :T, time, steam: baking temperature, baking time and steam used during the 2 nd baking phase of par-baking) (Debonne et al., 2017)*

	V (mL)	W (g)	Spec. V ratio (mL/g)	Crust thick. (mm)	L*	a*	b*
Intercept	150.6	80.5	1.334	-4.1	87.7	-9.6	-26.8
T	0.57	-0.08	0.014	0.03	-0.02	0.05	0.26
time		-0.62	0.162	0.46	2.77	-1.60	0.73
steam		-0.04		0.01	-0.00		
T*time		0.00		-0.00	-0.02	0.01	
T*steam		0.00		-0.00			
time*steam		0.00		-0.00			
T*time*steam		-0.00		0.00			
R²	0.17	0.91	0.33	0.77	0.84	0.92	0.64

Bread spoilage


Breads are most likely to be spoiled by fungal species of the genera of *Penicillium* spp. or *Aspergillus* spp. However, bread spoilage due to chalk yeasts (also called chalk moulds) is a concern as well. Chalk yeasts are characterized by the ability to spoil foods by means of the formation of powdery and filamentous colonies due to the fragmentation of hyphae into short lengths. Their appearance resembles a lot like moulds. Therefore, these are also often called *chalk moulds*, although being yeasts. There are three main chalk moulds, *Sacharomycopsis fibuligera* (formerly known as *Endomyces fibuliger*), *Hypopichia burtonii* and *Wickerhamomyces anomalus* (cfr. *Pichia anomala*). In order to prevent the spoilage of bread due to yeasts and mould spoilage, it is important to fully comprehend the influence of environmental conditions on the growth. Therefore, the growth behaviour of bread moulds (*P. paneum* and *A. niger*) and yeasts (*S. fibuligera*, *W. anomalus* and *H. burtonii*) is mapped through both *in-vitro* as *in-vivo* assays (plate assays and shelf-life and/or challenge tests) (Debonne et al., in progress).

Natural bread preservation

Essential oils

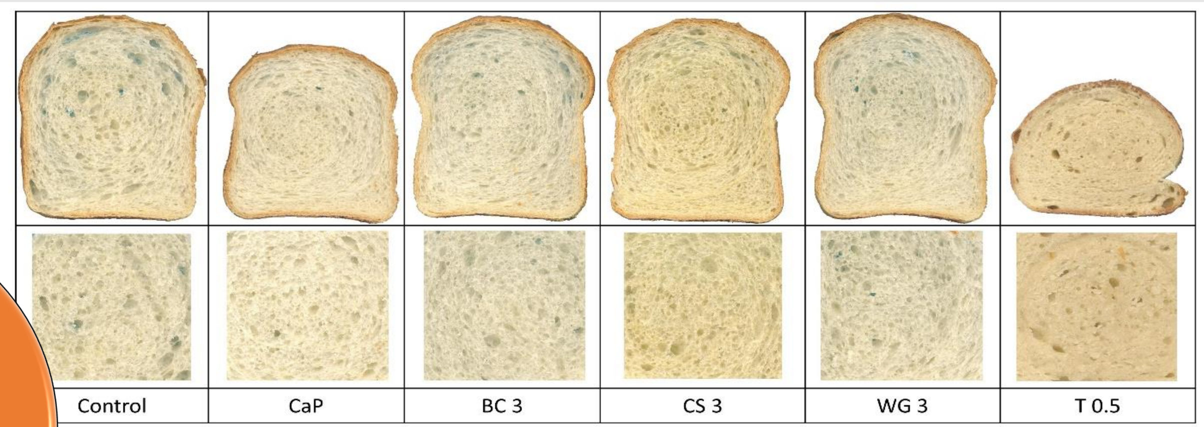



Figure 1 Pictures of bread slices and close-up of the crumb structure of the bread slices baked with : calcium propionate (CaP 0.2 g / 100 g flour), blackcurrant (BC 3 mL / 100 g flour), cumin seed (CS 3 mL / 100 g flour), wheat germ (WG 3 mL / 100 g flour) and thyme essential oil (T 0.5 mL / 100 g flour) compared to the control bread (Debonne et al, in progress)

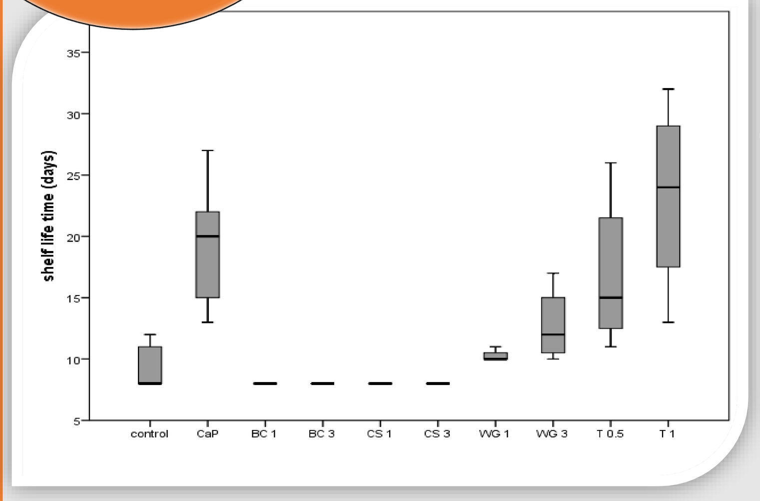
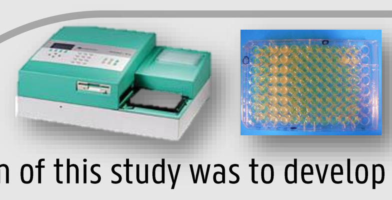
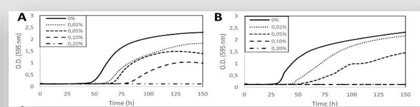


Figure 2 Visible mould free shelf-life time (days) for all breads baked with: calcium propionate (CaP 0.2 g / 100 g flour), blackcurrant (BC 1 or 3 mL / 100 g flour), cumin seed (CS 1 or 3 mL / 100 g flour), wheat germ (WG 1 or 3 mL / 100 g flour) and thyme essential oil (T 0.5 or 1 mL / 100 g flour) compared to the control bread (: significantly different from the control (p < 0.05)) (n = 12) (Debonne et al, in progress)*

Essential oils can exert antimicrobial activity. However, the use of EOs in bakery products is rather limited due to the inhibitory effect on yeast and its strong organoleptic effect (Debonne et al., 2018).

Antifungal screening

The aim of this study was to develop a fast, reproducible and accurate micro-dilution turbidimetric screening method for the antifungal activity of low water-soluble substances, e.g. essential oils (EOs). The influences of water activity (a_w 0.88, 0.93, 0.95 and 0.97), pH (4.8, 5.0, 5.5 and 6.0) and room temperature (22 and 30 °C) were discussed, and were correlated with results of the macro-dilution method. As a result, the minimal inhibitory concentration (MIC) of thyme EO (*Thymus zygis*) in a semi-solid medium on the growth of *Penicillium paneum* was determined. This study shows that results of the optimized method are comparable with results of the macro-dilution method regarding the influence of a_w, pH and temperature and thyme EO. Growth at a_w 0.88 was significantly reduced compared to a_w 0.93, 0.95 and 0.97. The modelled MIC-values ranged between 0.89 and 3.52 µL/mL. This study also showed the growth behavioral effect of *P. paneum* at pH 6. The micro-dilution method proved to be a rapid, accurate and reproducible method which can be used for the screening of antifungal activity of potential water or oil soluble extracts.

Table 2 Minimal inhibitory concentrations of thyme essential oil (µL/mL) with their 95% confidence limits (LL: lower limit; UL: upper limit) on growth of Penicillium paneum in function of pH (4.8, 5.0, 5.5 & 6.0), a_w (0.93, 0.95 & 0.97) and temperature (22 & 30 °C) (Debonne et al., in progress)

		aw 0.93	LL	UL	aw 0.95	LL	UL	aw 0.97	LL	UL
4.8	22 °C	2.25[a,1]	1.74	2.82	2.96[a,1]	2.10	4.44	1.14[a,2]	1.04	1.26
	30 °C	1.16[b,c,1]	1.03	1.32	2.40[a,2]	2.08	2.80	1.31[a,b,1]	1.14	1.53
5.0	22 °C	1.12[b,c,1]	1.01	1.24	2.74[a,2]	2.06	3.76	1.70[b,c,3]	1.51	1.93
	30 °C	1.39[b,1]	1.13	1.72	2.43[a,2]	2.01	2.99	2.85[c,d,2]	1.91	4.54
5.5	22 °C	2.59[a,1]	2.14	3.12	3.30[a,1]	2.37	4.88	1.81[c,2]	1.58	2.10
	30 °C	0.89[c,1]	0.75	1.09	2.65[a,2]	2.14	3.37	1.61[a,b,c,3]	1.24	2.10
6.0	22 °C	3.52[a,1]	2.78	4.43	2.03[a,1]	1.69	3.43	2.77[d,1]	2.26	3.45
	30 °C	*	/	/	2.40[a,1]	2.03	2.89	2.64[c,d,1]	2.00	3.64

[a-c] Values in the same column followed by different letters differ significantly (p < 0.05)
[1-2] Values in the same row followed by different numbers differ significantly (p < 0.05)
* No model value

Sourdough

Sourdough is often used as a natural preservative in bread production. In Debonne et al. (2018), the antifungal effect of sourdough on growth of *Penicillium paneum* Frisvad (IHEM 6652) and *Aspergillus niger* (P1118) was shown. The use of sourdough resulted in promising shelf-life extending properties. The antifungal activity of sourdough was hypothesized to result from the formation of organic acids, such as lactic and acetic acid, other fermentation products or can result from the activity of yeasts in the sourdough. In both *in-vitro* as *in-vivo* assay, pH and aw showed no significant effect on growth of *A. niger* and *P. paneum.* Therefore in a further study, the influence of the mole ratio of lactic acid over acetic acid (FQ, fermentation quotient) within the range of 0 – 4 will be explored in both in-vitro assays and through challenge and shelf-life tests.

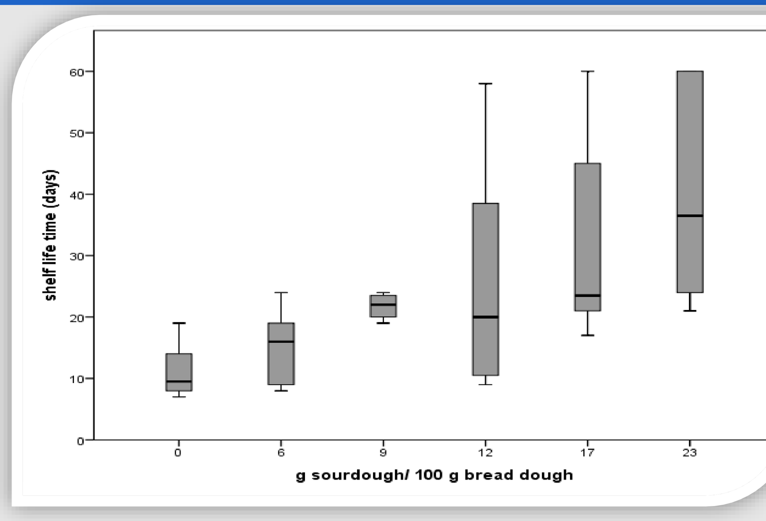


Figure 3 Boxplot of shelf-life (days) of MA-packaged wheat bread with sourdough (0, 6, 9, 12, 17 and 23 g/ 100 g bread dough, with respective pH values of 5.73 ± 0.06, 5.32 ± 0.25, 5.21 ± 0.11, 5.03 ± 0.14, 4.81 ± 0.32 and 4.72 ± 0.20) (n ≥ 8). All breads were stored at 22 °C (Debonne et al, 2018)

Table 3 Data of quality parameters of par-baked bread with sourdough (g SD / 100 g bread dough): volume (V, mL), pH and aw on days 0 and 3 and bread crumb color (L, a*, b*) (n > 6) (Debonne et al, 2018)*

Sourdough	V (mL)	pH0	pH3	Aw0	Aw3	L*	a*	b*
0	226.7 ± 16.6[a]	6.01 ± 0.17[a]	5.76 ± 0.12[a]	0.968 ± 0.015[a]	0.958 ± 0.006[a]	77.90 ± 4.13[a]	1.12 ± 0.41[a]	18.48 ± 0.72[a]
15	234.0 ± 24.2[a]	5.07 ± 0.21[b]	4.83 ± 0.06[b]	0.957 ± 0.006[a]	0.952 ± 0.006[a]	76.31 ± 2.55[a]	2.19 ± 0.95[a,b]	18.41 ± 3.33[a]
30	205.7 ± 5.5[a]	4.78 ± 0.09[c]	4.61 ± 0.04[c]	0.955 ± 0.010[a]	0.955 ± 0.003[a]	78.62 ± 1.00[a]	2.19 ± 0.06[b]	17.14 ± 1.94[a]

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Conclusion

The increasing interest in *clean label* food products has heightened the need for natural antimicrobial preservation strategies. In bread and other bakery products, the replacement of chemical preservatives such as propionates and sorbates is of particular interest as there are increasing signs of negative effects due to the intake of chemical preservatives and the changing consumer perception towards food preservatives and E-numbers. In our study, potential natural preservatives are pre-screened with an optimized reproducible screening method for both fungi and yeasts occurring on bread (e.g. essential oils, fermentation products, etc.). The bread moulds and yeasts are subjected to a thorough study in order to fully comprehend their mode of action and to tackle the problem of spoilage. Further, the results are validated on an actual bread matrix through both shelf-life and challenge tests. Additionally in this study, the role of the par-baking process and subsequent storage on technological, sensorial as well as microbiological food quality is explored.

Contact

Els.Debonne@ugent.be
PhD student / Assistant at Ghent University
www.ugent.be

Universiteit Gent
@ugent
Ghent University


GHENT UNIVERSITY

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bellona patis
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