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ASSESSMENT OF THE ANTIFUNGAL PRESERVATION POTENTIAL OF NATURAL WATER OR OIL SOLUBLE COMPOUNDS IN BOTH IN-VITRO AND BREAD BAKING TRIALS

Introduction

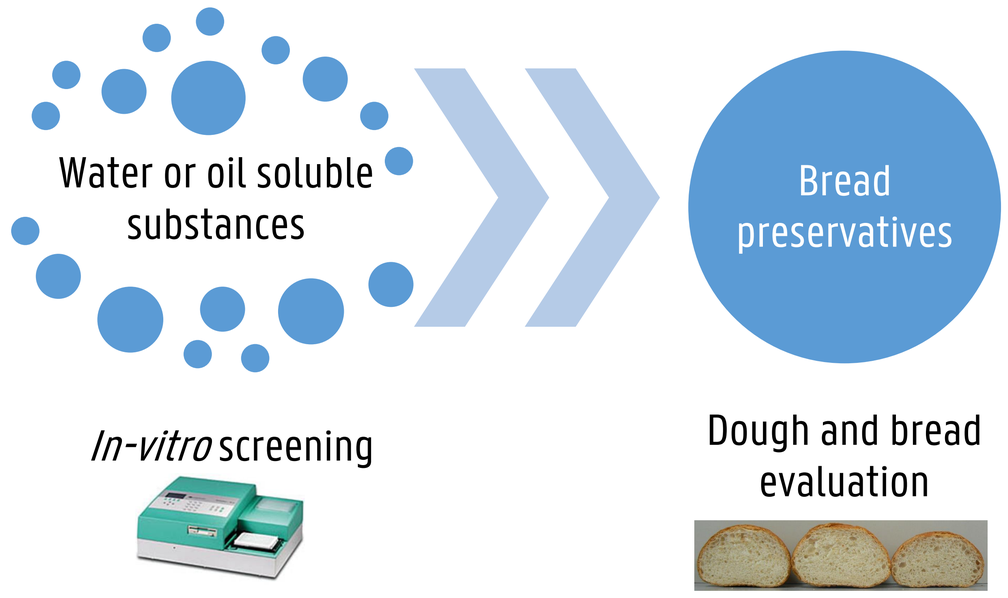
Mould spoilage of bakery products is a serious problem resulting in large numbers of food waste. The food industry is interested in replacing chemical preservatives by natural alternatives in order to meet up with the consumers' changing wants and needs as well as with the *clean label* trend. Therefore, this study focusses on exploring natural water or oil soluble compounds with antifungal activity in the bread matrix to reduce fungal growth of primarily *Penicillium* spp. and *Aspergillus* spp..

Goal 1

Antifungal *in-vitro* pre-screening techniques generally consist in preparing a water-based-agar or broth. In addition, to investigate low water-soluble compounds such as oils, very often solvents or detergents are added to increase the stability of the emulsion. However, this may decrease the antimicrobial activity of the tested compounds. As a consequence, a micro-dilution screening method was developed that could screen both water and oil soluble substances, based on the measurement of fungal growth in a semi-solid yeast extract sucrose (YES) medium through optical density.

Goal 2

Furthermore, the natural compounds were tested in actual dough and bread systems, assessing the technological quality of dough and bread, such as dough strength, dough rise, bread volume, colour and bread texture. In addition, the antifungal activity of the compounds was investigated throughout shelf-life and challenge tests.



Methods

Antifungal screening with the YES medium

The semi-solid medium with adjusted pH and a_w was suitable for the screening of antifungal activity of water or oil soluble components (example: thyme essential oil), A 96-well microtiter plate with a dilution series of the component was prepared and placed in a Multiskan Ascent 96/384 Plate Reader, Optical density was measured every 20 min during 6 days. The Time To Detection (TTD) was recorded and was the time corresponding with an OD-increase of 0.1.

Growth / no-growth modelling

The growth/no-growth data were used to develop G/NG models for *Penicillium paneum* with pH, a_w, concentration of thyme oil as example and incubation time as explanatory variables, incubated at 22 or 30 °C. An ordinary logistic regression model was used to describe the data. The models were developed in SPSS through backward stepwise regression and were plotted in Matlab.

Dough and bread evaluation

The influence of the natural components on dough and bread quality was evaluated, Dough characteristics such as flour water absorption, mixing properties of dough, starch gelatinization and dough rise gradient, together with bread quality, such as specific volume, colour, crumb and crust texture, and antifungal activity in bread through both shelf-life and challenge tests were determined.

Results

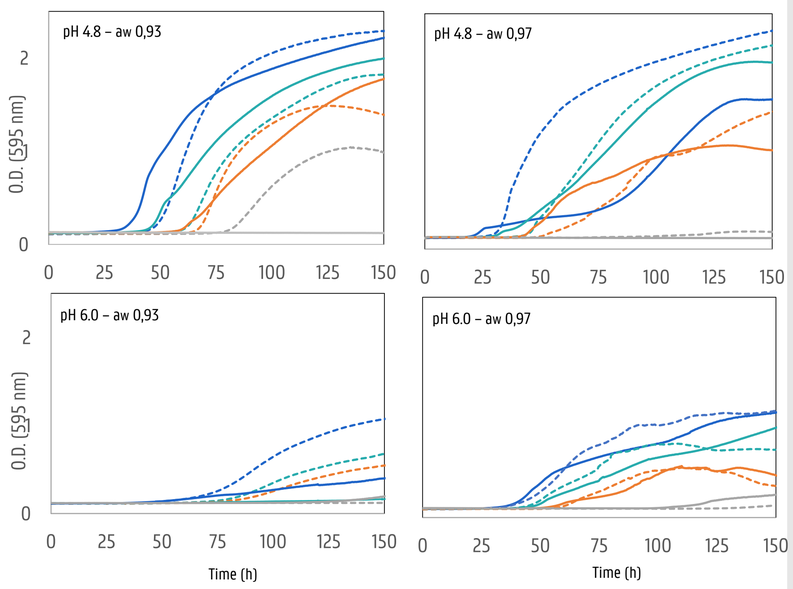


Fig. 1 Average growth curves (8 individual curves) showing growth of *Penicillium paneum* obtained at various combinations of pH, a_w & thyme essential oil: 0 µL/mL (blue), 0.2 µL/mL (green), 0.5 µL/mL (orange) & 1.0 µL/mL (grey). Incubated at 22 (striped lines) and 30°C (full line)

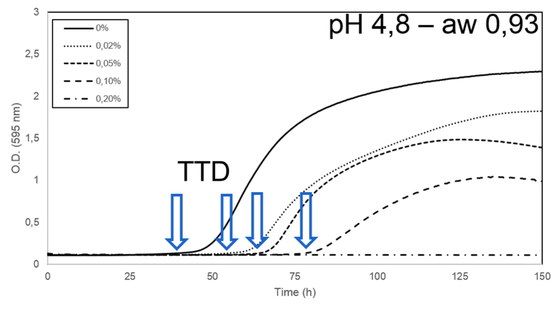


Fig. 2 Illustration of the Time To Detection (TTD) given on the growth curves of *P. paneum* incubated at 22 °C with pH 4.8 and aw 0.93.

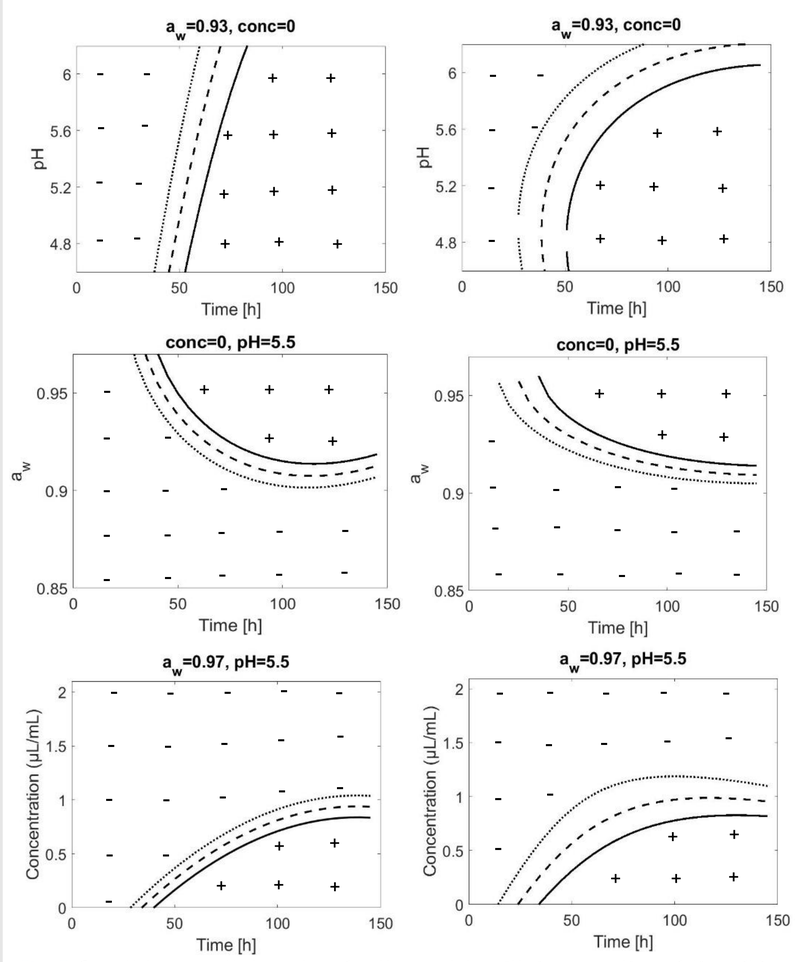


Fig. 3 Growth/no-growth boundary for the models with incorporation of time (h), with different environmental conditions (pH, aw, thyme essential oil and temperature (1st column: 22 °C; 2nd column: 30 °C)) as a function of time. Lines represent the ordinary logistic regression model predictions $p = 0.9$ (—), $p = 0.5$ (- -), $p = 0.1$ (...) (+: growth area; -: no-growth area)

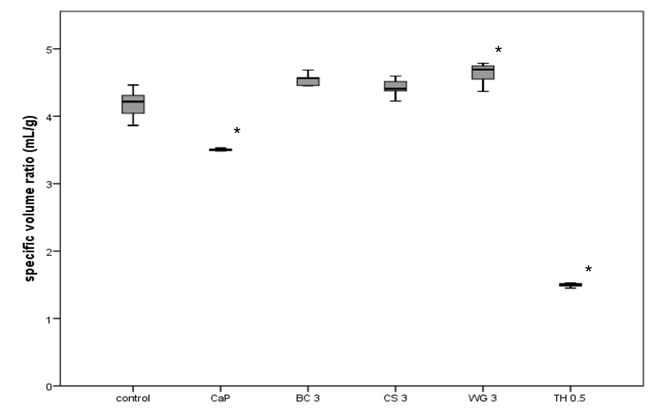


Fig. 4 Specific volume ratio (mL/g) measured 2h after baking for all breads baked with calcium propionate (CaP 0.2 g/100 g flour), blackcurrant (BC 3 mL/100 g flour), cumin seed (CS 3 mL/100 g flour), wheat germ (WG 3 mL/100 g flour) and thyme essential oil (T 0.5 mL/100 g flour) compared to the control bread (n = 6).

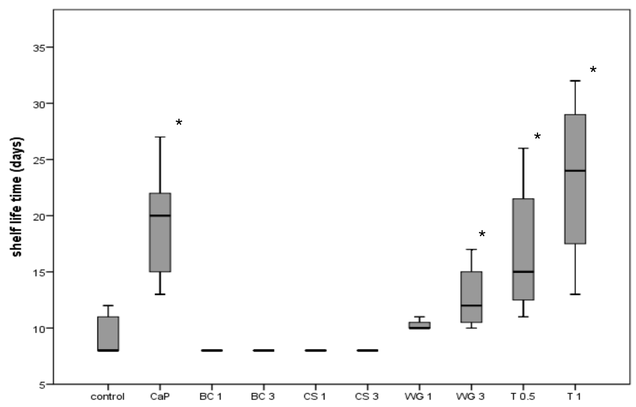


Fig. 5 Visible mould free shelf-life (days) for all breads baked with calcium propionate (CaP 0.2 g/100 g flour), blackcurrant (BC 1 or 3 mL/100 g flour), cumin seed (CS 1 or 3 mL/100 g flour), wheat germ (WG 1 or 3 mL/100 g flour) and thyme essential oil (T 0.5 or 1 mL/100 g flour) compared to the control bread (n = 12)

Conclusion

The antifungal screening method based on the growth of fungi in a semi-solid YES-medium is suitable for all kinds of water or oil soluble substances, provided that solutions of these substances are clear. To pre-screen the antifungal activity of natural substances to be later used as an ingredient of a model food matrix or an actual food matrix, it is important to use a dilution test for mimicking the activity when dissolved or dispersed in the matrix. The micro-dilution method overcomes issues of phase separation in case of oil testing and provides a rapid screening of multiple substances, at different concentrations, and under various incubation conditions (pH, a_w, temperature). Moreover, the use of growth/no-growth models can give more information on the interaction effects of environmental growth conditions Therefore, this predictive modelling based on optical density data is the perfect step-up for *in -vivo* trials which are necessary to overcome matrix effects and to fully understand the mode of behavior of the substances in a food matrix as well as to include influences of the components on the structural and sensorial properties of food products. The results for the effect on dough and bread quality show that the process of finding suitable substances showing both antifungal activity as well as a good behavior in the final bread product is challenging, however necessary due to the changing consumer perception towards traditional chemical preservatives.

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