ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

for Food & Function article **"Enhancing the nutritional profile of regular wheat bread while maintaining technological quality and adequate sensory attributes"**

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Microbiological Shelf Life and Water Activity of Reference Wheat Bread (RWB) and High-Protein Hybrid Bread (HPHB)

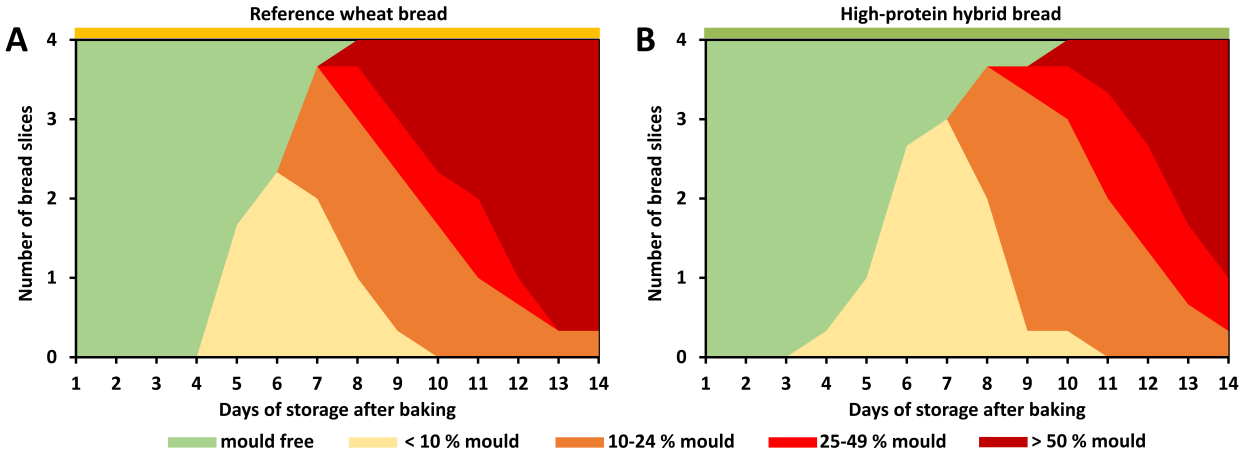

Fig. 1 Microbiological shelf life of (A) RWB and (B) HPHB as indicated by ambient air challenge test. Results represent the mean of three independently performed challenge tests.

Results and Discussion

In addition to crumb staling, the shelf life of bread is affected by microbial deterioration. While also bacteria and yeast can cause bread spoilage, a contamination with fungal spores from the bakery environment after baking is considered the most common reason.[1] Mold growth typically shows a positive correlation with water availability in the food product; the critical water activity, however, varies with fungal species, temperature and substrate.[2] Apart from an unpleasant visual experience for consumers, mould spoilage can cause the formation of off-flavours, allergenic compounds and mycotoxins, potentially even before visibility of fungal growth.[3] It also leads to a substantial amount of food waste - in UK households an estimated 20 % of bread goes to waste due to mould growth.[4,5] Therefore, susceptibility to mould deterioration represents a food safety hazard and indicator for economic loss and should be considered when bread quality is evaluated. The microbial shelf life of both bread formulations was monitored in an ambient air challenge test. The results are presented in Figure 1. A slight tendency towards earlier onset of mould growth for HPHB was observed. The results also suggest a deceleration of mould growth in HPHB represented by later onset of stages 3 - 5 (10 to $>$50 % of slices covered in mould). However, these tendencies cannot be considered significant differences and the experiment generally indicated a similar microbial shelf life of HPHB and RWB. This observation can be supported by very similar water activities measured for both formulations (RWB 0.945 ± 0.003, HPHB 0.943 ± 0.003).

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Materials and Methods used for the Determination of Microbiological Shelf Life and Water Activity

Microbiological shelf life of the breads was evaluated using an ambient mould challenge test as described by Dal Bello *et al.* [6] with some modifications. Bread loaves where sliced in a sterile manner to obtain four slices of 20 mm thickness per loaf. Instead of a treatment with conidial solutions of fungi, each slice was microbiologically challenged by exposure to the bakery ambient air for 5 min on each side. The slices were separately packed in sterile plastic bags which were heat sealed. To guarantee comparable aerobic conditions in all bags, a filter pipette tip was inserted. During a storage period of 14 days (at room temperature), mould growth was visually evaluated. Based on the percentage of slice area covered with fungal growth, slices were sorted into five categories as follows: 0 % - mould free, $<$10 % mould, 10-24 % mould, 25-49 % mould, $>$50 % mould. Four slices were monitored from each of three batches per formulation. Water activity of the fresh bread crumb was measured using a water activity meter (HygroLab, Rotronic, Basserdorf, Switzerland).

Abbreviations

The following abbreviations were used:

HPHB	High-protein hybrid bread
RWB	Reference wheat bread

References

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