PE8.30 Growth of Yeasts in five modified Atmosphere packed Meat Models 341.00

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***Abstract* - Growth of the yeasts *Debaryomyces hansenii*, *Candida zeylanoides*, *Candida sake, Pichia guilliermondii,* and *Yarrowia lipolytica* was measured in five different meat models containing nitrite and/or lactate or glucose. The meat models were packed in modified atmosphere (MA) and stored at 8 °C for up to 6 weeks. The growth of *C. zeylanoides* was reduced by nitrite whereas the other yeasts were not affected or grew even better when nitrite was added. *Yarrowia lipolytica* were inhibited by lactate. The yeasts grew to high numbers during storage and could reduce the shelf life of the meat products by producing a diverging odour in the packages or by producing carbon dioxide which could lead to "blown packs".**

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***Index Terms* - growth, lactate, meat model, nitrite, spoilage, yeast.**

I. INTRODUCTION

Meat products are often stored at low temperatures in low oxygen/high carbon dioxide atmosphere, and hurdles as nitrite and lactate are added to reduce spoilage from bacteria. However, these conditions may favour the growth of yeasts in meat products. The occurrence of different yeasts in the micro flora of meat products has been investigated recently [3] but limited knowledge is available on the growth of yeasts in meat products containing traditional hurdles. The aim of the present study was to evaluate the effect of nitrite and lactate on growth of yeasts which have shown a potential for growth in a meat model system [3].

II. MATERIALS AND METHODS

A. Yeasts

Yeast strains used were *Debaryomyces hansenii* (DMRICC 4863), *Candida zeylanoides* (DMRICC 4867), *Candida sake* (DMRICC 4868), *Pichia guilliermondii* (DMRICC 4889), and *Yarrowia lipolytica* (DMRICC 4861). The yeasts were maintained, prepared for inoculation and enumerated as previously described [3].

B. Meat model substrate

The pork sausages were produced, inoculated, and packaged as previously described [3]. The pork sausages had the following composition: shoulder, 51.9% (w/w); jowls, 18.0% (w/w); ice-water, 21.2% (w/w); potato starch, 3.7% (w/w); soya isolate, 1.4 (w/w); caseinate EM-HV, 1.4% (w/w); food-grade sodium polyphosphate N 15-15, 0.3% (w/w); sodium chloride, 2.0% (w/w). Some batches of meat sausages were added 150 ppm Na-nitrite, or 2.0% (w/w) Purasal S (Na-lactate content 58-61%) (Purac, The Nederlands) or both. To one batch 1.0% (w/w) dextrose was added. All additives came from SFK (Avedøre, Denmark). The meat sausages were pasteurized to a core temperature of 75 °C. The five different meat models contained the additives in the following manner as shown in Table 1.

Meat model	Na-Lactate (%)	Sodium-nitrite (ppm)	Glucose (%)
I	-	-	-
II	-	150	-
III	1.2	-	-
IV	1.2	150	-
V	-	-	1.0

Table 1. Distribution of additives in the five meat models.

C. Packages and storage

Two identical slices of meat sausage packed with a gas consisting of 20% CO_2, 0.5% O_2, and 79.5% N_2. The packages were stored at 8 °C for up to 6 weeks. For some of the samples, the packages were penetrated by a needle after 4 weeks of storage to measure growth in atmospheric air.

D. Chemical and microbiological analysis

The NaCl concentration, water percentage, fat percentages and pH were analyzed as previously described [1], sugar content and head-space gas composition were analyzed as previously described [3]. L-lactate was analyzed by an UV method (Boehringer Mannheim Cat. No.10 139 084 035 R- Biopharm AG, Darmstadt) according to the manufacturers manual.

Yeasts were enumerated after serial dilution on MYGP plates incubated at 20 °C for 5 days. When the packages were opened for microbiological analysis, the technician recorded the odour olfactometrically and compared the odour with an un-inoculated control.

III. RESULTS AND DISCUSSION

The chemical composition of the five meat models is shown in Table 2. Regarding pH, the content of water, fat and sodium chloride, the composition of the meat models are quite similar to normal commercial Danish heat treated meat products with the exception of the nitrite content. As shown in Table 2, about 110 ppm residual nitrite was present in meat model II and IV after heat treatment. To Danish meat products only 60 ppm of nitrite are added, the larger addition was made to make it easier to evaluate the effect of nitrite. The 0.4 % L-lactate found in meat model I, II and V originates from the raw meat material. Addition of 2 % Purasal resulted in a final concentration of L-lactate of 1.9 %, which is within the normal application level [4]. The glucose found in meat model I-IV must originate from the ingredients for example the starch.

To some extent, all five yeasts could grow in all meat models at 8°C in the modified atmosphere packages. However, when the packages were opened after 4 weeks, additional growth was seen in all samples (Fig. 1 A-E). The high oxygen concentration probably allows an improved yield on the available nutritious substances. With atmospheric air the “hurdles” nitrite and lactate or addition of glucose had little or no impact on the yeast counts after 5 weeks at 8 °C, at least for *D. hansenii*, *C. zeylanoides*, and *P. guilliermondii* (Fig 1 A, B, and D).

Addition of 150 ppm nitrite to the MA-packed meat model affected the growth of all five yeasts in different ways. The growth of *C. zeylanoides* was inhibited by addition of nitrite in meat model II and IV (Fig 1 B), whereas an increase in growth was seen with *D. hansenii* when nitrite was added (Fig 1 A). *D. hansenii* can utilize nitrite as a nitrogen source [5]. A difference in the tolerance towards nitrite has recently been shown between *C. zeylanoides* and *D. hansenii*. *Debaryomyces hansenii* was more tolerant to the inhibitory effect of acidified nitrite at pH 5.5 than *C. zeylanoides*. For this organism, the effect of nitrite was partly explained by the uncoupling effect of energy generation from growth [2]. *Pichia guilliermondii* and *Y. lipolytica* seem to have a slightly faster growth rate for the first 2 weeks when nitrite was added compared with the control samples (Fig 1 D and E). Addition of nitrite did not have an effect on growth of *C. sake* (Fig 1 C).

Addition of 2 % Purasal alone to the MA-packed meat model reduced the growth of *D. hansenii* slightly (Fig 1 A) whereas *C. zeylanoides, C. sake,* or *P. guilliermondii* (Fig 1 B-D) were not affected. For *Y. lipolytica* some reduction in growth was observed in meat model III and IV compared with the control samples (Fig 1 E).

Addition of dextrose to the meat model did not have any effect on the growth of the yeasts except for *P. guilliermondii* for which the growth was slightly higher after 2 weeks compared with the control, and for *C. sake* higher cell counts were found in the meat model with glucose added (Fig 1 C).

The head space gas contained between 0.67-0.80 % O_2 and 13.7-15.2 % CO_2 on day 1 in un-inoculated samples. In the un-inoculated samples, the O_2 increased to 0.96-2.23 % and the CO_2 decreased to 9.3-13.3 % after 6 weeks of storage due to diffusion through the top film of the packages. The gas composition of the inoculated samples corresponded to the observations from the growth curves.

For *D. hansenii* the O_2 content in samples from meat models containing nitrite was between 0.026-0.079 % after 2 weeks whereas the other three models contained between 0.252-0.627 % indicating faster growth and oxygen consumption in the samples containing nitrite. After 6 weeks, the O_2 content was between 0.012-0.031 % and the CO_2 between 14.5-15.9 % in all samples.

For *C. zeylanoides* the O_2 content in the samples containing nitrite (meat model II and IV) was between 0.702-0.722 % after 2 weeks whereas the other 3 models contained between 0.005-0.016 % O_2 indicating inhibited growth of *C. zeylanoides* in the samples containing nitrite. After 6 weeks, the head space O_2 content was between 0.004-0.061 % and CO_2 between 13.9-15.6 % in all samples.

Candida sake grew fast in all meat models, and after 2 weeks the O_2 concentration in the head space was below the detection limit of 0.001 %, and the CO_2 concentration was increased to between 19.7-36.8 %. After 4 weeks, the CO_2 concentration peaked in the meat model containing glucose with a CO_2 content of between 40.0-40.2 % in the head space, the four other meat models contained between 19.3-23.2 %. The O_2 concentration was below the detection limit in all samples after 4 and 6 weeks.

Similar results were found in samples inoculated with *P. guilliermondii*. All meat models had a O_2 content under the detection limit after 4 and 6 weeks. The CO_2 concentration after 6 weeks was increased to between 17.0-23.3 % with the highest concentration in the meat model containing glucose.

For *Y. lipolytica,* after 6 weeks of storage, the samples from the meat models containing lactate had an O_2 content of 0.663-0.846 % whereas the other three meat models had a content between 0.034-0.511 % indicating inhibition of growth in the samples containing lactate.

The samples were evaluated olfactory metric when the samples were opened for microbiological analysis. The samples inoculated with *D. hansenii* were described as having an odour of soy sauce or beer, with *C. sake* the odour was described as pungent whereas the odour of samples with *P. guilliermondii* was described as yeast and bread. The samples with *Y. lipolytica* had a stale odour. No distinct odour was recorded for the samples inoculated with *C. zeylanoides*.

IV. CONCLUSION

All yeasts in this investigation could grow in the meat model system even when traditional hurdles as nitrite or lactate were present. *C. zeylanoides* were inhibited by nitrite but the other yeasts were not affected or grew even better when nitrite was added. *Yarrowia lipolytica* were inhibited by lactate but the other yeasts were not affected much. Addition of glucose increased the growth of *C. sake*, but the other yeasts were not affected by addition of glucose. *Pichia guilliermondii* and *C. sake* could reduce the oxygen content of the head space gas below the detection level of 0.001 % oxygen and could produce carbon dioxide which could lead to "blown packs". Some of the yeasts produced a diverging odour in the samples that would reduce shelf life of the products. These results show that yeasts may cause problems in meat products that are preserved with traditional hurdles to reduce or prevent growth of bacteria.

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REFERENCES

[1] Jacobsen, T., Budde, B.B., & Koch, A.G. (2003) Application of *Leuconostoc carnosum* for biopreservation of cooked meat products. Journal of Applied Microbiology, 95, 242-249.

[2] Mortensen, H.D., Jacobsen, T., Koch, A.G. & Arneborg, N. (2008) Intracellular pH homeostasis plays a role in the tolerance of Debaryomyces hansenii and Candida zeylanoides to acidified nitrite. Applied and Environmental Microbiology 74(15), 4835-4840.

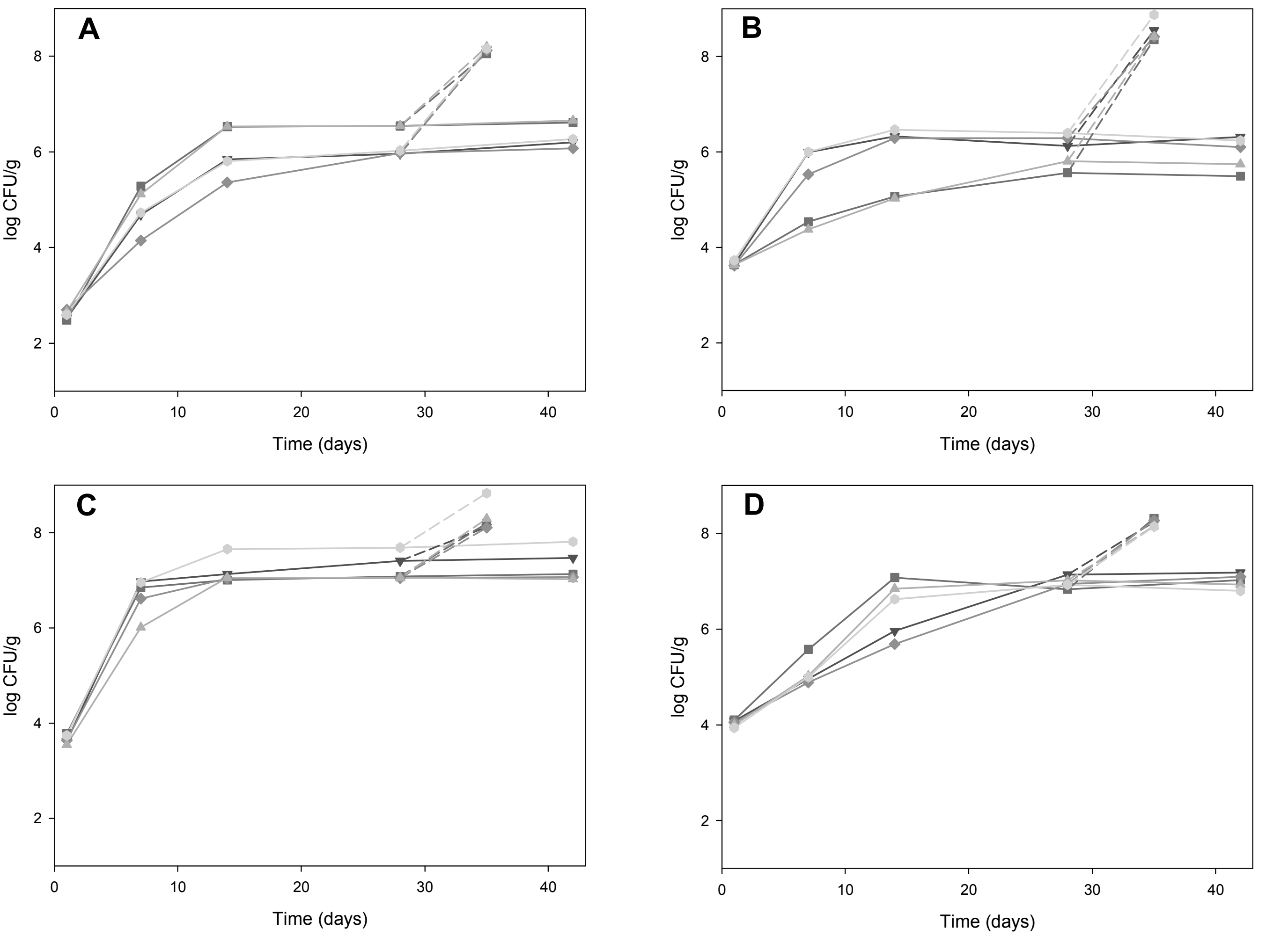
[3] Nielsen, D.S., Jacobsen, T., Jespersen, L., Koch, A.G., & Arneborg, N. (2008). Occurrence and growth of yeasts in processed meat products – Implications for potential spoilage. Meat Science, 80, 919-926.

[4] Søltoft-Jensen, J., & Hansen, F. (2005). New chemical and biochemical hurdles. In Da-Wen Sun, Emerging technologies for food processing (pp. 387-416). London: Elsevier Academic Press.

[5] Vigliotta, G., M. Di Giacomo, E. Carata, D. R. Massardo, S. M. Tredici, D. Silvestro, M. Paolino, P. Pontieri, L. Del Giudice, D. Parente, & Alifano, P. (2007) Nitrite metabolism in *Debaryomyces hansenii* TOB-Y7, a yeast strain involved in tobacco fermentation. Applied Microbiology and Biotechnology, 75, 633-645.

Meat model	pH	Water (%)	Fat (%)	NaCl (%)	Na-nitrite (ppm)	L-lactate (%)	D-Glucose (%)
I	6.3 ± 0.1	63.6 ± 0.3	16.7 ± 0.1	2.14 ± 0.02	0	0.4 ± 0.0	0.2 ± 0.0
II	6.3 ± 0.1	63.6 ± 0.3	16.6 ± 0.4	2.13 ± 0.03	108 ± 4	0.4 ± 0.0	0.2 ± 0.0
III	6.3 ± 0.1	63.5 ± 0.2	15.6 ± 0.2	2.08 ± 0.03	0	1.9 ± 0.1	0.2 ± 0.0
IV	6.3 ± 0.1	62.9 ± 0.0	16.3 ± 0.3	2.06 ± 0.02	109 ± 8	1.9 ± 0.1	0.2 ± 0.0
V	6.3 ± 0.1	63.7 ± 0.7	15.9 ± 0.9	2.08 ± 0.01	0	0.4 ± 0.0	1.1 ± 0.1

Table 2. Chemical composition of the five meat models after heat treatment. All measurements were made in duplicate. The results are means ± standard deviation of 2-3 different batches of the five meat models.



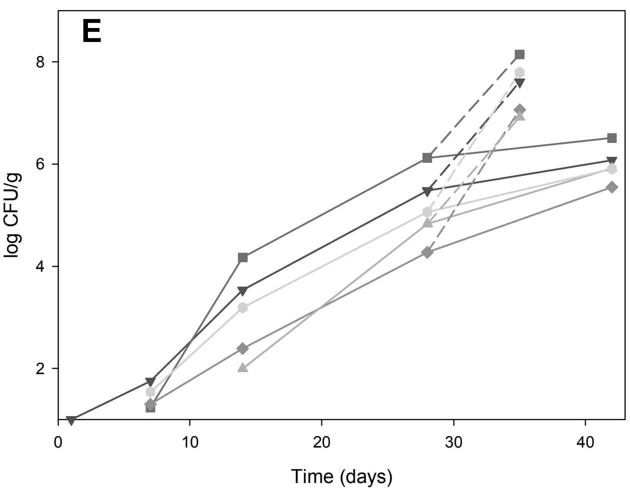


Figure 1. Growth of 5 yeast species in 5 different meat models at 8 °C in modified atmosphere packages ((0.5% O_2, 20% CO_2, 79.5% N_2) solid line). Packages with all yeasts and meat models were opened after 28 days of incubation, and further incubated for 7 days before growth was measured (dotted line). (A) *Debaryomyces hansenii*, (B) *Candida zeylanoides*, (C) *Candida sake*, (D) *Pichia guilliermondii*, and (E) *Yarrowia lipolytica*.