SUSCEPTIBILITY OF CEREAL MICROFLORA TO OXYGEN DEFICIENCY AND CARBON DIOXIDE CONCENTRATION

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Abstract

Susceptibility to oxygen deficiency of grain microflora was investigated in order to quantify the possibilities and limits of airtight storage of wet grains (0.85 < a_w (water activity) < 0.98). Experiments were conducted with naturally contaminated rice, in experimental micro-silos with carefully controlled atmospheric compositions.

At moisture contents not allowing bacterial growth (i.e. a_w < 0.87), most storage fungi (Aspergillus, Penicillium) are fully inhibited in atmospheres containing less than 1 % oxygen. Results showed that if this low partial pressure is reset daily, simulating a leak in an airtight silo, yeasts (Candida spp.) and a yeast-like fungus, Aureobasidium pullulans, are able to develop even with less than 0.5 % O_2, and the higher the a_w, the more rapid the growth. On the other hand, if O_2 is completely lacking (under 100 % CO_2 or N_2 or completely airtight conditions), no fungal growth -neither sporulation nor mycelial growth- can be observed, regardless of the a_w. At a_w higher than 0.90, viz., a moisture content permitting bacterial activity, the main phenomenon was the development of lactic bacteria, which could not be inhibited by any gaseous composition.

These experimental data conform closely to previous results obtained in pilot-scale experiments under airtight conditions. They show the bacteriological and mycological limits of controlled atmosphere storage of wet grains.

Introduction

At moisture contents not allowing bacterial developement and in particular not permitting the growth of facultative anaerobes such as lactic acid bacteria, it seems possible to store damp grain under modified atmospheres for several months, without drastic changes in technological properties. The most critical factor is the residual level of oxygen (O_2) ; whereas most storage fungi are fully inhibited in atmospheres containing less than 1 per cent O_2, certain yeasts or yeast-like fungi are known to be able to develop slowly, at very low O_2 tensions (Teunisson, 1954 ; Burmeister and Hartmann, 1966 ; Gonen and Calderon, 1968 ; Pelhate, 1982).

Such development was observed after 5 or 6 months of airtight storage of corn at 21 % moisture content in hermetic metallic silos. After about 1 year, the population of yeasts (Candida spp.) had

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reached a high level but the grain was still usable for animal feed as well as for the starch industry (Richard-Molard et al., 1984). Nevertheless, the grain had undergone some biochemical changes, acidification and a sour odor of fermentation for example, which could pose a problem if the grain were to be used for human consumption. Furthermore the aspects of possible pathogenicity or toxinogenicity of these yeasts developping on wet cereals during airtight storage are not well known.

The aim of experiments reported in this paper was to study the microbial evolution of damp cereals (paddy) under modified atmospheres with very low quantities of available O_2. These very low quantities simulate leaks in hermetic silos or porosity of materials like cement or plastics often used or proposed for hermetic storage. At the same time, effects of concentrations of carbon dioxide (CO_2) were investigated in order to determine whether or not this gas can act as an inhibor for microorganisms in airtight storage of grains.

1. Materials and methods

1.1. Materials

All experiments were conducted in strictly hermetic micro-silos (10 l capacity) with commercial paddy rehumidified at water activities of 0.94 and 0.87. Under each condition, the natural microflora of paddy was studied, but in addition grains were artificially inoculated during rehumidification by a toxigenic strain of *Aspergillus flavus*.

1.2. Modified atmospheres

Modified atmospheres were obtained either by just sealing the micro-silos (airtight conditions) or by flushing, when full, with compressed gases, pure or after mixing in order to obtain the required composition. CO_2 concentrations were measured by I.R. absorption (Binos CO_2-meter) and O_2 partial pressures were monitored with a magnetodynamic-meter. It must be pointed out that with such an O_2-meter it is necessary to take into account the negative paramagnetic effet of CO_2 when low partial pressures of O_2 are determined against high concentrations of CO_2.

1.3. Analytical methods

Bacteria, yeasts and molds were evaluated by dilution methods, using plate count agar for mesophilic aerobes, Rogosa agar for *Lactobacillus spp.* and 2 % malt agar for yeasts and molds. Fungal growth was also estimated by ergosterol determination, using an HPLC procedure described elsewere (Cahagnier et al., 1983) and aflatoxin B_1 was measured by HPLC with fluorimetric detection.

2. Results and discussion

2.1. Evolution of microflora of paddy at 25 % moisture content (m.c.) under strict anaerobic conditions

Strict anaerobic conditions were established in micro-silos in two different ways : by airtight storing the wet grains or by flushing it with pure CO_2 before sealing the micro-silos. Under airtight condition, O_2 was consumed within one day by high m.c. grain and a concentration of about 90 % CO_2 was naturally obtained after about 20 days.

Figure 1 shows the corresponding microbial development. Only lactic acid bacteria (i.e. Lactobacillus spp.) were able to develop under these modified atmospheres and as expected they produced evident but slight changes in odor of the grain. It should be noted that an atmosphere of pure CO_2 did not inhibit bacterial growth at this m.c. On the other hand, mold counts decreased continuously during the three months of the experiment and the ergosterol level remained constant. But probably the most interesting result lies in the fact that under these particular conditions of strict anoxia, the growth of yeasts and yeast-like fungi was not possible, whereas it was evident in pilot scale experiments at lower m.c. (Richard-Molard et al., 1984).

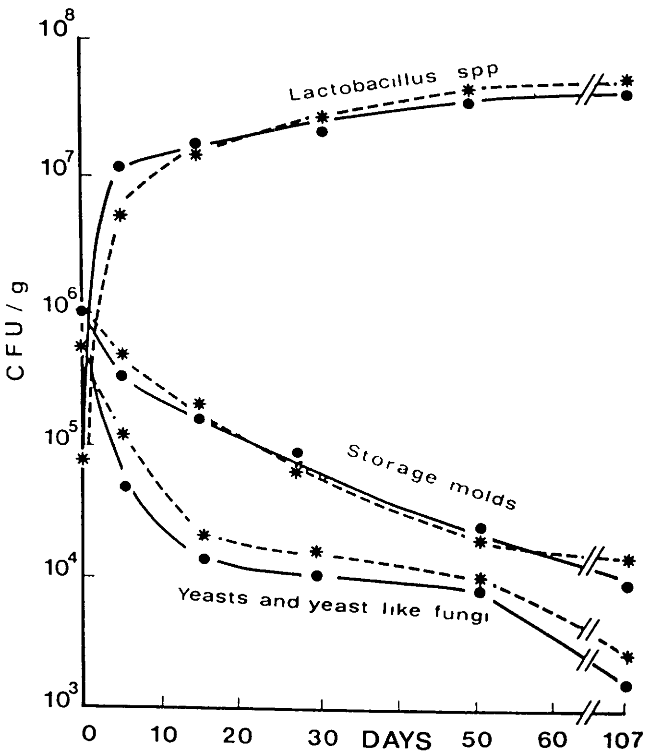

Figure 1 : Microbial development on paddy stored under strict anoxia at a_w 0.94, under pure carbon dioxide (●), and in airtight conditions (✱).

2.2. Effect of CO_2 concentrations on yeasts and molds growth

Investigations on the possible inhibitory effect of CO_2 on yeasts and molds were carried out on grains at 18 - 19 % m.c. in order to avoid extensive growth of lactic acid bacteria.

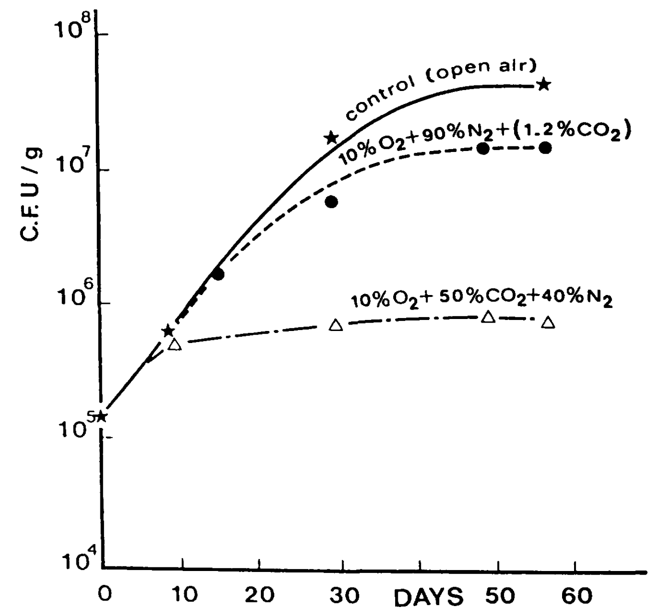

Figure 2 : Effects of carbon dioxide and restricted oxygen on xerotolerant molds in paddy at a_w 0.87

As shown in Figure 2, these investigations were undertaken with O_2 concentration restricted to 10 % supplemented either with pure nitrogen, or with 50 % CO_2 and 40 % nitrogen.

The modified atmospheres were reset every day by flushing the micro-silos during a few minutes. In comparison with the control sample stored in the open air, in which xerotolerant molds developed rapidly, producing about 65 micrograms of ergosterol after two months, the limiting effect of restricted O_2 was evident, especially on mycelial growth as estimated by ergosterol content (Figure 3). In fact, under all conditions, the growth rate of molds was the same at initiation of experiments and limitations appeared only after 10 to 20 days, when the O_2 demand of the fungal populations increased in relation with increased production of biomass. A 50 % CO_2 together with O_2 restricted to 10 % led to a drastic inhibition of storage molds which produced only 5 micrograms of ergosterol within two months.

Nevertheless, under these conditions, an important development of yeasts (Candida sp.) and yeast-like fungi (Aureobasidium pullulans) was also observed up to 10^7 CFU/g, whereas no growth of these microaerophilic organisms could be observed when no CO_2 was introduced into the modified atmosphere. Thus the real effect of high concentrations of CO_2 is not clear since yeasts and molds were probably in competition for oxygen, and it would be necessary to experiment with grains contaminated only with pure strains of molds, to completly elucidate the role of CO_2 on fungal growth in airtight storage of cereals.

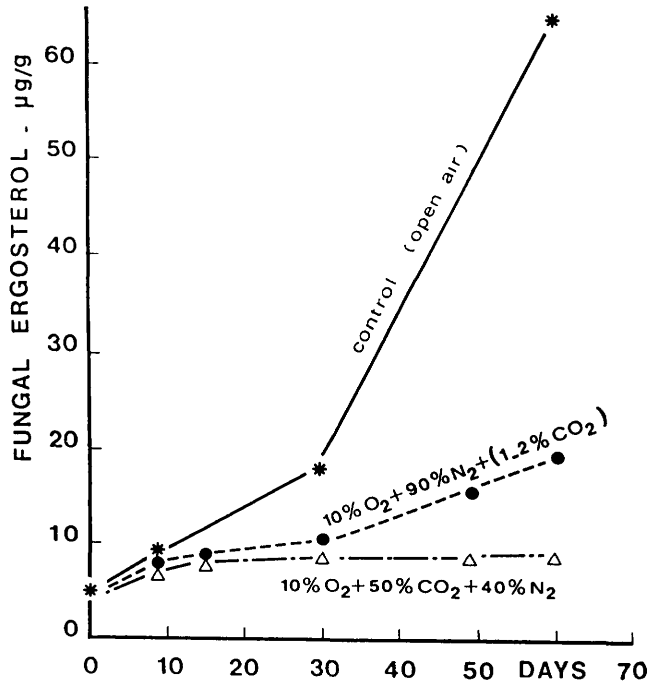

Figure 3 : Effects of carbon dioxide and restricted oxygen on mold growth estimated by ergosterol content of paddy (a_w 0.87)

2.3. Influence of extremely low oxygen levels

In the first section of this paper it was shown that when a strict anoxia was applied to wet grains, no growth of storage yeasts occurred whatever the m.c. of the grain. During pilot scale experiments, it is always very difficult to quantify the rate of O_2 penetration into hermetic silos due to leaks or porosity of materials, because of the strong O_2 demand of wet grains. Therefore, microbial development described on grains said to be stored under "about 1 % residual O_2" for example are very often difficult to interpret.

Results presented in Figures 4 and 5 were obtained with grains stored at a_w 0.94 and 0.87, under extremely restricted O_2. Every day, the micro-silos were rapidly flushed with CO_2 containing 0.5 and 1 per cent of O_2, as indicated by the O_2-meter. After corrections, it

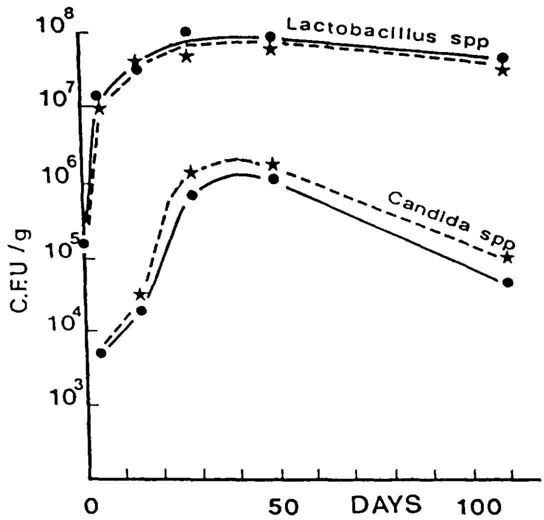

Figure 4 : Influence of very low oxygen levels on microbial development of paddy at a_w 0.94
(★) : 1 % O_2 + 95 % CO_2 ; (●) : 0.5 % O_2 + 95 % CO_2 (see text)

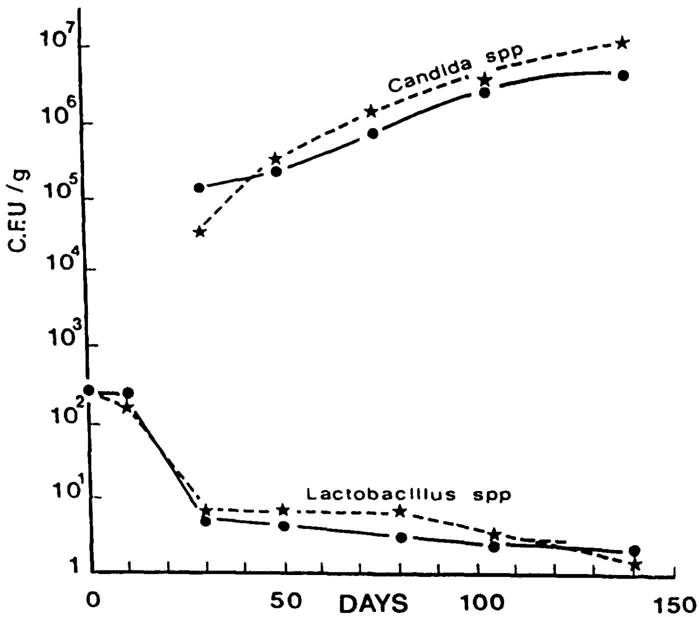

Figure 5 : Influence of very low oxygen levels on microbial development of paddy at a_w 0.87
(★) : 1 % O_2; (●) 0.5 % O_2 + 95 % CO_2 (see text)

was calculated that about 15 and 30 mg of O_2 respectively (i.e. about 2 and 4 micrograms of O_2 per of grain) were introduced into the silos each day.

Under these particular conditions, as expected, molds disappeared (results not shown) within two months at a_w 0.94 and four months at a_w 0.87, and no aflatoxin B_1 was produced. Lactic acid bacteria grew extensively at the higher m.c. but were not able to develop at a_w 0.87. On the other hand, O_2 is necessary for the development of yeasts but extremely low levels are sufficient to allow the growth of Candida sp. (tentatively identified as Candida variabilis (Lind, Berkout) and Aureobasidium pullulans, leading to off-odors formation in the grain. Yeasts developped more slowly, but more extensively at a_w 0.87 than at a_w 0.94, possibly due to the absence of bacterial growth at this m.c.

Conclusions

These experimental data are consistent with previous results obtained in pilot scale experiments. They clearly show the bacteriological and mycological limitation of controlled atmosphere storage of wet cereal grains. At m.c.s permitting bacterial activity (i.e. at $a_w > 0.90$), the main phenomenon is always the development of lactic acid bacteria which cannot be inhibited by any gaseous composition.

On the basis of the present results, it remains difficult to say if high concentrations of CO_2 are inhibitory for molds or stimulating for yeasts, when some O_2 is available. The growth of yeasts on grains of intermediate m.c. is not possible under strict anaerobic conditions but very low levels of O_2 are sufficient to initiate such a development. When yeasts develop, strong modifications of the odor of the grain are always observed whereas lactic acid bacteria seem to be able to produce only slight changes in the organoleptic properties of the grains.

Our conclusions in this field is that it would probably be possible to store wet grain up to about 20 % m.c. without any significant microbial change if completely hermetic structures could be used.

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