PILOT SCALE EXPERIMENTS ON HALF-WET MAIZE STORAGE UNDER AIRTIGHT CONDITIONS: MICROBIOLOGICAL AND TECHNOLOGICAL ASPECTS

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ABSTRACT

Fungi of cereal grains are generally recognized to be strictly aerobic. Several recent publications indicate that these fungi can grow to some extent even under very low oxygen tensions. In laboratory scale experiments, we have shown that it is possible to store grains at 21% moisture content in hermetic structures (w/w) for several months without appreciable changes in microflora. Accordingly, a pilot-scale experiment was carried out in a hermetic mini-silo of about 1 m^3 of capacity, at the same moisture content. Analytical results show that in such conditions, a natural anaerobiosis is obtained within 4-5 days (O_2 0.5%) after a very slight alcoholic fermentation. Subsequently, during a six months period of storage the technological and nutritional qualities of the grain remain unchanged. During the next six months the lactic bacteria and the yeast show weak, but noticeable, metabolic activity and growth. In spite of this unexpected microbiological growth the qualitites of the grain were still acceptable after one year of storage. Microbiological analysis show that mould counts were very low at the end of the experiment, no mycelial growth occured (checked by determination of ergosterol in grains) and no mycotoxins were produced. This grain storage technique seems therefore of interest because of the increasing cost of drying. A new experiment is now being conducted on a larger scale. Same preliminary results are presented.

INTRODUCTION

During the last 10 years, the cost of maize drying, harvested in France with a moisture content between 35 and 45% wb, has increased very much. In order to minimize that cost, several processes have been developed. At first energy-saving drying technologies have been evolved: dryeration, high temperature dryers, two-step dryers (MULTON and LASSERAN, 1979). But, generally speaking this tendancy has been to increase the temperature of incoming air. This has led to a decrease of the quality of maize, due to the thermal shock; a problem of particular concern in the case of maize used for starch production. As alternatives two wet storage techniques have been developed: (i) the "silage", which is mainly lactic fermentation, exclusively used for cattle feed, and (ii) the "cribs", a natural and slow drying process. Unfortunately, the slowness allows moulds to grow and produce toxins, when the climate is not appropriate. Wet storage using propionic acid is too expensive and irradiation is not satisfactory.

Noting all these disadvantages, we have studied in our laboratory a method which consists in partially drying the maize to 20–22% mc and storing this 'half wet' maize in air tight silos. It appears that between one third and one half of the cost of drying may be saved, and the thermal degradation avoided.

A previous study conducted in our laboratory using a small quantity of grain (1kg) (Richard-Molard *et al.*, 1980) has shown that in such conditions of humidity and airtightness, the oxygen of air trapped inside the sealed box is quickly converted to CO_2 by the respiration of moulds and the grain itself. Generally speaking the higher the moisture content and the temperature, the quicker is this phenomenon. When the partial pressure of O_2 has become very low (0.5%) respiration is stopped and moulds decrease and disappear progressively, killed by anaerobiosis. According to the literature, at that moisture content (22%), the water activity is too low to allow development of bacteria: only some yeasts can grow, but very slowly. Therefore all living processes are stopped and good preservation of grains is guaranteed.

Then the question was to check if the same effects could be observed with grain in bulk, and what are the changes in technological, nutritonal and microbiological qualities under these conditions. Two pilot-scale trials described here, were carried out to observe these changes. If these experiments were successful it appeared to us that the technique had potential for large scale application to the storage of partially dried grains destined for industries where the first step of the process is to temper the grain and which require grain without thermal degradation. These are mainly the starch industry and the cattle feed industry, making pellets and granules.

PILOT EXPERIMENT IN SMALL METAL SILOS (1 m^3)

Experimental procedure

Five 1 m^3 metal sealed bins, specially built for these experiments were completely filled with maize (one with dry maize, as reference, and four with half-wet grain).

The openings on the top for filling and at the bottom for emptying, were closed by screwed metal cover plates, the airtightness of which was ensured by a rubber gasket.

Three small tubes, closed by a valve, and connected to a device measuring the concentration of O_2, were fitted at the top, the bottom and centre of each bin. Three temperature probes were also arranged along the axis of the cell. These silos were placed inside the pilot plant area of the Research Centre, so that the temperature variations of external air were largely reduced and no noticeable thermal gradient could occur.

Maize

The maize used in this trial (unknown variety) was grown in Nantes area, (Western coast of France, Atlantic climate). After harvesting at about 40% moisture content, it was gently dried at a moderate temperature (80°C) to 21.5% moisture content in a discontinuous farm dryer.

METHODS

Moisture determination

Standardized method AFNOR NF-V-03-708, identical to ISO-6540, ICC 110-1 and OIML (No. 8) has been used (Multon, 1982).

Microbiological determination

Under anaerobic conditions, moulds which are supposed to grow or to metabolise, cannot sporulate (Tabak and Cooke, 1968). So counting conidia by the classical suspension-dilution method cannot be used to demonstrate a possible mould activity. This method has nontheless been used as a control for anaerobiosis. To some extent, the "Ulster" method (Musket and Malone, 1940) takes into account mycelial growth but is not really quantitative. For this reason the mycological analysis has been complimented with a gas-chromatographic determination of specific volatile compounds which indicate a fungal activity and with the high pressure liquid chromatography analysis of fungal ergosterol to show mycelial growth.

Enumeration of bacteria

The classical microbiological methods were employed for enumeration of the following bacteria groups: general mesophilic bacteria (PCA), Enterobacteriaceae (VRBG), Lactobacilli (Rogosa Agar), Anaerobic (TGY* Agar) and especially sporulated Clostridia.

Yeasts and moulds

The suspension-dilution method was used for yeast counts (Cahagnier, 1973). For the Ulster method (malt extract agar), 400 grains were analysed each time, 200 grains being superficially disinfected, 200 grains being not. Disinfected grains lead to aberrant results, probably due to increasing permeability of stored kernels (Pelhate and Theriault, 1979). For this reason those results are not considered here.

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* TGY : Trypticose, Glucose, Yeast extract agar.

Collection and analysis of volatile organic compounds

The volatiles were desorbed under vacuum, collected in a dry-ice cooled trap and recovered with methylene chloride. The extract was then concentrated by solvent evaporation and analysed by gas-chromatography, using a glass capillary column (50m length, 0.5mm id) coated with carbowax 20m. The oven temperature was programmed from 70°C to 150°C at a rate of 2°C/minute with helium as carrier gas (Richard-Molard *et al.*, 1976).

Fungal ergosterol assay

Fungal ergosterol was extracted with methanol from grounded kernels, saponified with KOH, extracted from methanol with petroleum ether, purified and analysed by HPLC using a 5u-Spherisorb Column (Seitz *et al.*, 1977) Cahagnier and Poisson, 1982).

Mycotoxins

The mycotoxins were determined with the multidetection method of Stoloff (1971).

O_2 concentration

O_2 concentration in intergranular atmosphere of the silo was determined directly with a paramagnetic O_2 meter (La Gazometrie **) working on a sample of air, which was pumped out and reinjected into the silo.

DETERMINATION OF TECHNOLOGICAL QUALITY

Grain fitness for industrial starch extraction. The industrial starch value of maize was estimated by extraction in a laboratory pilot plant, according to method described by Beaux and Le Bras (1982). The efficiency of extraction and quality of extracted starch was estimated through 13 different tests.

Nutritional quality

The nutritional value was estimated, as a first aproach, with growth trials, carried out on pullets. Two homogeneous groups of 24 pullets were fed for 4 weeks with a feed made of 22% soybean, 6% mineral and vitamins and 72% maize (experimental stored maize for one group, and the same maize but dried immediately after harvest, for the other group). Nutritional value was assessed by the quantities ingested and by the increase in weight of the pullets.

** La Gazometrie, 33 rue Antoine Marie Colin, 94400 Vitry-sur-Seine, France.

Biochemical parameters

Very classical methods were used for biochemical determinations:

- for starch content: Ewers' method
- for protein content: Kjedhal method (N x 625)
- for lipid content and ash: standardized EEC method
- for fat acidity: standardized AFNOR method (extraction in 95% ethanol, neutralization by KOH/ethanol, with phenolphthalein as indicator; results in mg H_2SO_4/100g dry matter.)

RESULTS AND DISCUSSION

Changes in temperature, moisture content and dry matter. Since the experimental silos were situated inside the pilot plant, the influence of outside temperature variation was not significant, and the temperture of the grain (17°C) did not change during the storage period. Table 1 shows that the moisture content and dry matter (measured by weight of 1000 grains) did not change either.

Table 1 - Moisture content and dry matter as a function of time.

Storage time Days	0	34	93	150	280	326
Moisture content (% wb)[a]	21.28	21.20	21.70	21.73	21.67	21.80
Dry matter[b] grams	243.3	241.5	241.9	238.5	237.6	240.6

a average of 12 samples

b dry weight of 1000 grains

SPEED OF DECREASE OF O_2 CONCENTRATION

Theoretical calculation. It is possible to compute, theoretically, the consumption of O_2 and the time necessary for the concentration of O_2 to decrease to zero. The full silo, if internal volume ca $0.93 m^3$, contained 690 kg maize. As 40% of the apparent volume is occupied by intergranular atmosphere, the total volume of air existing inside the silo is, including a small head space, about $0.400\ m^3$. The total volume of pure oxygen is then:

$$0.4 \times \frac{21}{100} = 84 \text{ litres.}$$

There are

$$\frac{84}{22.4} = 3.75 \text{ moles}$$

of O_2 inside the silo (neglecting correction of temperature).

With respect to the stochiometric equation of starch oxidation (and assuming as an approximation, that maize is constituted only of starch):

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + \text{energy}$$

one can say that for each mole of O_2 used, there is one mole of CO_2 produced. Therefore, at the end of the process, when all oxygen is used, there are 3.75 moles of CO_2 in the silo, ie a mass of:

$$3.75 \times 44 \simeq 165\text{g of } CO_2 \qquad (1)$$

According to Srour (1982) the rate of production of CO_2 (q) in bulk grain is given by the following equation:

$$q = k \exp(a\theta) \qquad (2)$$

where q is expressed in mg CO_2/100 g dry matter/day, θ is final temperature in ^{o}C, k is a constant, being a function of moisture content and species (for maize at 21.5%, $k \simeq 4$), and a is a constant, being à function of the type of grain (for cereals, $a = 0.1385$).

Under the conditions of our experiment (final $\theta \simeq 17^{o}$C), we compute that the speed of production of CO_2 resulting from respiration is given by

$$q = 4.\exp(0.1385 \times 17) \simeq 42.13\text{mg } CO_2/100 \text{ g dry matter/day}$$
$$= 421 \text{ g } CO_2/\text{tonne dry matter/day.} \qquad (3)$$

The silo contained 0.69 tonnes of maize at 22% moisture content, ie 0.54 tonnes of dry matter. Therefore the total speed of production of CO_2 inside the silo was 227 g/day (3). From (1) and (3) it is easy to compute that all the oxygen might have been converted into CO_2 after about:

$$\frac{165}{227} = 0.77 \text{ day.}$$

EXPERIMENTAL DETERMINATION

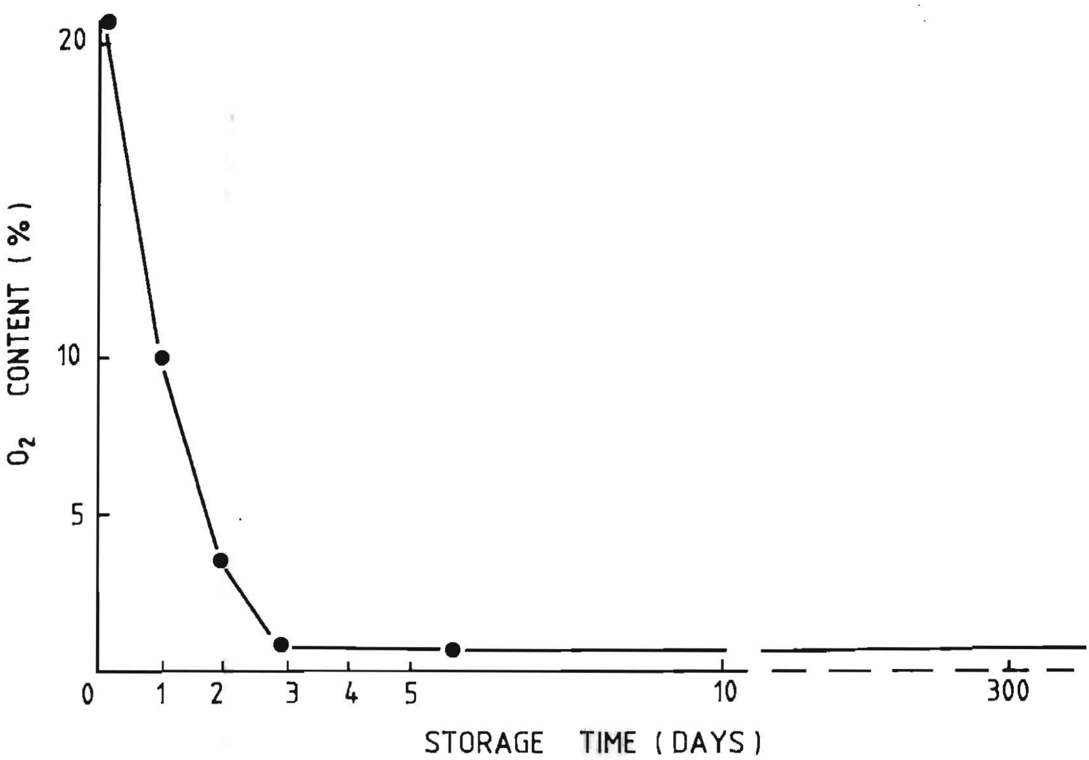

Figure 1 - Decrease of O_2 concentration inside the silo as a function of time

Figure 1 shows the decrease of O_2 concentration as a function of time of storage. It is clear that O_2 concentration is lower than 0.5% in about 3 days, 3 times longer than predicted by the theoretical calculation. This difference is certainly due to the fact that the equation (2) is representative of CO_2 production in aerated conditions. In confined conditions the metabolism and therefore the CO_2 production rate (or oxygen consumption) decreases progressive as the CO_2 increases.

This confirms the concept of the storage and shows the system is well sealed.

MICROBIOLOGICAL ACTIVITIES AND METABOLISM DURING STORAGE

Micro-organism growth

The results obtained are shown on Figure 2. We observed a continuous decrease of the number of mesophilic and aerobic, facultative anaerobic bacteria growing on TGY medium. In contrast there was pronounced development of *Lactobacillus* reaching a level of 3.3 x 10^4 germs/gramme (g/g) after 5 months, but decreasing thereafter.

The yeasts decreased from the beginning of the trial, but after 5 months, some species of *Candida* were growing.

Moulds, when estimated by simple counting, showed a very important, and rapid reduction of the total number, except for the *Mucorales*, which remained constant for about 1 month.

The 'Ulster method' gives a semiquantitive estimate of the mycelium growth of the different species. It was noted particularly that the fields species (*Epicoccum, Cladosporium, Verticillium, Cephalosporium*) disappeared very quickly. Fusarium remained a longer time on the grains, but disappeared completely before the end of the trial.

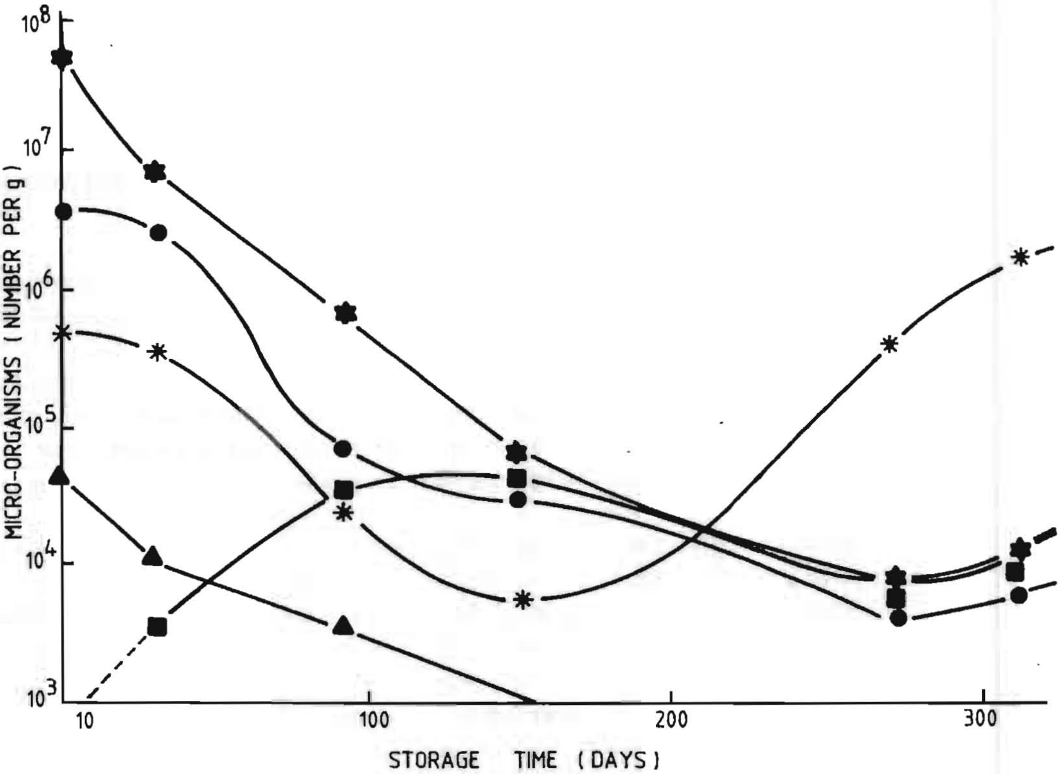

FIGURE 2 - MICROBIOLOGICAL EVOLUTION OF MAIZE, AS A FUNCTION OF STORAGE TIME.
MESOPHILIC BACTERIA
OPTIONAL ANAEROBIC BACTERIA
LACTOBACILLUS
YEAST
MOLDS

Storage fungi, sparsely represented by some *Penicillium*, also disappeared very quickly, except again the *Mucorales*, characteristic of an intermediate flora well adapted to the lack of oxygen (Pelhate, 1978). The genus *Rhizopus* seemed particularly tolerant, but did not grow in the conditions of experiment. Anyway, at the end of the trial, when the silos were emptied, the grain was free of bacteria and moulds and only yeasts were present. The external appearance of this maize was excellent.

Ergosterol content

The biosynthesis of ergosterol requires oxygen (Weete, 1973). It is a good indicator of mould development (Cahagnier *et al.*, 1983). The average ergosterol content (four determinations) obtained from each silo was not significantly different (1.1, 1.09, 1.08, 1.07, 1.06 and 1.1 µg/g of grain).

The initial value is typical of a late harvested grain, and the constancy of this value during storage shows clearly that (i) there was no fungal evolution, and (ii) no entry of oxygen had occured during the trial. In fact, a small quantity of oxygen could have entered without causing a measurable increase of the concentration of oxygen in the silo since moulds used O_2 at the same speed as it comes in. However, in that case, ergosterol production would appear as a consequence of the mould activity.

Changes in volatile compounds of intergranular atmosphere

Only certain specific volatile compounds have been looked for. No presence of oct-1-ene-3-ol, an indicator of mould growth, was observed.

Some traces of isoamyl alcohol and B-phenyl-ethanol indicated a small alcoholic fermentation during the first days of storage, which stopped after some days.

Volatile compounds thus produced, remained in the grain and gave them a slight and characteristic smell of fermentation.

Mycotoxins

All the titrations of mycotoxins (aflatoxin B, ochratoxin A, sterigmatocystine, zearalenone) during and at the end of storage were negative.

CHANGES IN BIOCHEMICAL CHARACTERISTICS OF THE GRAIN

Table 2 shows the changes in the 7 biochemical characteristics which have been considered during this experimentation.

It can be seen that no important changes of these biochemical characteristics occurred during storage, except for the fat acidity, which increased slightly but continuously. This could be due to the lipase activity

Table 2 Changes in the main biochemical characterisitics as a function of time of storage.

	Storage Time (days)					
	0	34	93	150	280	326
Fat acitidy	0.025	0.031	0.037	0.062	0.069	0.079
Dry matter	84.8	84.5	85.1	84.2	83.3	83.6
Proteins % (d.b.)	9.36	9.38	9.60	9.64	9.50	9.30
Lipids % (d.b.)	4.62	4.65	4.52	4.89	4.68	4.87
Starch % (d.b.)	74.1	74.9	76.0	74.2	74.3	76.1
Cellulose % (d.b.)	2.5	-	3.8	3.3	2.5	2.7
Mineral Matter	1.10	1.30	1.20	1.20	1.35	1.25

Table 3 Changes in sugar fraction as a function of time (expressed in gramme/100 grammes of dry matter).

Time (days)	Sugars soluble in alcohol		Glucose	Fructose	Sucrose
	Orcinol	HPLC	H P L C		
0	1.25	1.30	0.08	0.06	1.00
93	0.51	0.56	0.13	0.15	0.18
150	0.52	0.53	0.20	0.23	0.10

It could be observed that sucrose is slowly hydrolysed into glucose and fructose during the experiment.

The lack of maltose and the ratio of glucose/fructose suggests that there was no noticeable amylolytic activity.

Table 4 Changes in starch value of maize as a function of time of storage (study made in starch pilot plant; results reported for a moisture content of 15%).

Test	Time of storage (in days)					
	0	34	93	150	280	326
Starch yield (% d.b.)	63.8	65.6	64.8	66.3	65.9	65.0
Starch recovery rate (%)	86.1	87.6	85.3	89.4	88.6	85.4
Protein content of starch (% d.b.)	.78	.63	.65	.58	.55	.61
Protein content of gluten (% d.b.)	27.0	28.9	27.4	27.8	27.2	26.7
Gluten yield	15.6	15.1	16.0	15.7	16.3	16.5
Protein recovery rate (%)	44.8	46.3	45.7	45.5	46.7	47.4
Filtration speed of gluten	12.0	13.1	12.2	11.7	11.9	11.6

TABLE 4 (continued)

Germ yield (% d.b.)	5.8	5.9	6.0	6.0	6.2	6.2
Fat content of germs (% d.b.)	56.2	56.3	55.2	56.1	55.0	54.6
Envelope yield	5.91	4.90	4.90	3.80	3.59	4.79
Turbidity	32.5	44.0	58.0	69.0	88.0	83.5
Sedimentation	58	77	72	67	93	78
Fitness to water tempering	39.8	39.9	40.3	40.4	39.8	40.5

of grains and micro-organisms, these enzymes being active in the abscence of O_2. More probably, such an increase is due to some organic acidity (eg. lactic acid, produced by microorganisms which was also extracted by 95% alcohol.

Nevertheless the fat acidity increase is rather small and does not indicate any significant alteration of the grain.

The changes in the sugar fraction (Table 3) are further evidence of a slight fermentation during the first month of storage, as suggested by microbiological data.

CHANGES IN TECHNOLOGICAL QUALITY

Starch value

The results of evaluation of starch values of the grain are given in Table 4.

When considering these results, the following observations could be made:

- the starch yield remained almost constant :
- grain stored for 326 days gave a starch as good as grain just harvested;
- the protein content of starch is similar to those obtained with a maize gently dried at 70-80°C;
- the fitness to water tempering, an important parameter to the starch processing industry, remains constant;
- the turbidity test showed a small deterioration of the protein : this change could be due to progressive acidification of the grains by organic acids (fermentation) leading to a partial insolubilization of proteins. Such a change has already been reported (Pelhate and Theriault, 1979 ; Carantino, 1979).

Nevertheless, generally speaking, the fitness of maize for industrial processing (as measured in a pilot extraction facility) remained very good throughout the storage period.

Nutritional value

The nutritional tests were made with selected pullets (homogeneous samples with respect to the weight, after four weeks ; during the first 12 days, the animals were fed *ad libitum* with a starting feed for chicks). The results of tests are reported in Table 5.

Table 5 Results of nutritional tests on pullets (R:for reference maize, S:for half-wet stored maize). Average values and standard deviation.

Storage Time (months)	5		11	
Diet	R	S	R	S
Dry matter ingested (g)	2,092.1 ($\pm$ 39.4)	2,179.6 ($\pm$ 31.6)	1,988.3 ($\pm$ 40.0)	1,907.9 ($\pm$ 44.9)
Weight increase	1,016.7 ($\pm$ 21.2)	1,119.5 ($\pm$ 17.1)	1,022.5 ($\pm$ 28.4)	1,011.2 ($\pm$ 26.2)
Nutritional efficiency (x)	0.496 ($\pm$ 0.004	0.514 ($\pm$ 0.003)	0.514 ($\pm$ 0.001)	0.530 ($\pm$ 0.047)

(x) Nutritional efficiency is the ratio of dry matter ingested over weight increase.

After 5 months of storage, the three tests showed better results for the hermetically stored grains than for the reference samples. The difference is highly significant. On the other hand, after 11 months, the results from the two first tests were slightly better for the reference maize, but the differences are very small and not significant for the weight increase and nutritional efficiency. Thus it appears clear that the hermetic storage of half-wet maize improves the nutritional value of maize during the first 5 months. After one year, the stored maize has the same nutritional value as the reference (fresh) maize.

Since the hermetically stored grain has a much better hygienic quality (absence of moulds and toxins) than crib-stored maize, this technique appears to be of great interest for the cattle feed industry.

STUDY, NOW IN PROGRESS, TO DEVELOP A STORAGE TECHNIQUE USING AIRTIGHT PLASTIC SILOS (10 m^3)

The experiments, reported above, have demonstrated the economic and scientific interest of airtight storage of half-wet maize. But it has been also emphazised that, in order to be successful, this method demands that the concentration of O_2 be kept always below 1 or, better, 0.5%. Therefore the sealed silo must be absolutely airtight. As the use of metal silos is probably too expensive for large industrial development, we have decided to check, in a small model (10 m^3), the reliability of a silo made with non-rigid, supple plastic sheets placed over a metal frame. Such structure could be 5 times cheaper than a simple metal silo.

For this trial, we collaborated with a French company, specialising in fabrication of plastic sheets, but hitherto mainly for military purposes and conditioned storage rooms for fruits (Societe Bachmann*).

Experimental procedure

An experimental silo, of approximately 10 m^3, pyramidal shaped, has been built and set up at Boigneville, in the I.T.C.F. experimental research station.

The plastic sheets were made of PVC. The permeability of the material is less than:

- for O_2 : 42.8 $cm^3/m^2/24$ h, at 20°C, under differential pressure of 100 g;
- for CO_2 : 284 $cm^3/m^2/24$ h, at 20°C, under differential pressure of 100 g;
- for H_2O : 12 $g/m^2/24$ h, at 38°C, with differential R.H. of 95%.

The roof was doubled inside the silo by a light plastic awning, separating the grain from the roof. This system creates a bed of air, isolating the grain from changes of temperature, and preventing water condensing on the roof from dropping on the grain. The plastic material used for the silo was chosen for its highly airtight/insulating properties and non-absorbancy of radiant energy. This experimental silo has been equipped with various accessories, as shown in Fig. 3.

* Bachmann Company : 69 rue Daniel Casanova, 94200 YVRY (France)

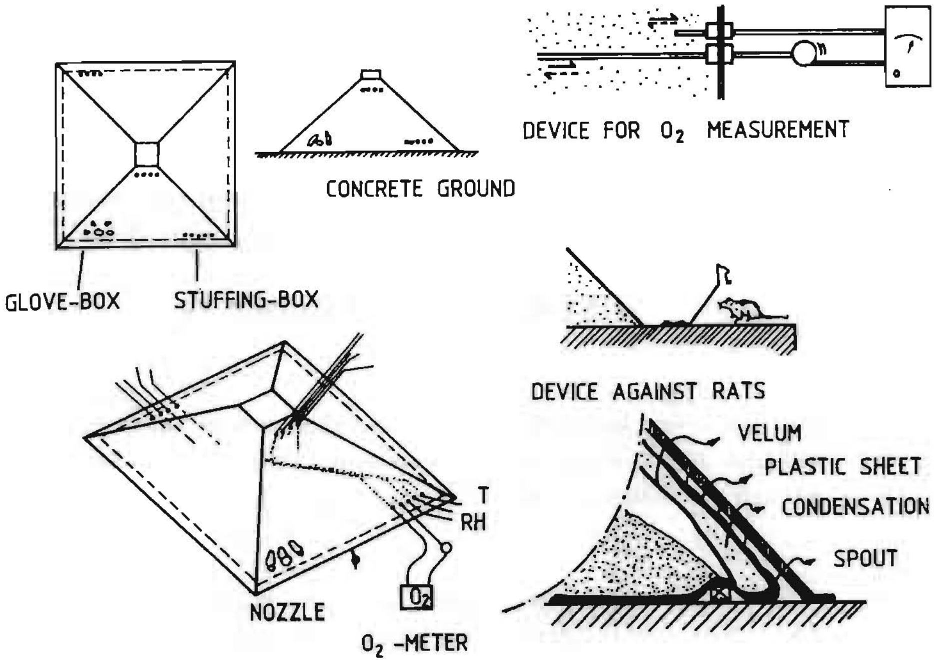

Figure 3. Arrangement of the different sampling systems in the experimental silo.

They are:

- a glove box and an air-lock allowing samples of grain to be taken without any introduction of oxygen (air) inside the silo ;
- temperature probes and relative humidity probes ;
- several tubes which could be connected to the device measuring the residual oxygen concentrations inside the silo ;
- some nozzles for removal of the water which eventually condensed and collected in the water receiver.

The maize used for this trial was grown on ITCF experimental fields. It was harvested at about 38% and then partially dried by ITCF to 19% moisture content.

The silo was filled at the end of November 1982.

Measurements were taken from each probe regularly once a week. When necessary, a sample of grain was taken for analysis.

Preliminary results and discussion

This trial, scheduled to run for 1 year, has now been in progress for 5 months. It is thus not possible to give complete conclusions for the trial but one can say that up to now it has been perfectly satisfactory.

The calculation used for prediction of the time taken for the oxygen to be removed can be used here. In this instance, with a final temperature of 5^{o} and larger volume, it is predicted that the process would take 9 days.

On two occasions an interesting incident occured : oxygen level was found to be too high (4 to 5%) and at the same time some mould development was observed. On inspecting the silo, a small leak was found in the stuffing box where probe wires pass through the wall. When this leak was sealed up by the technician, the level of oxygen very quickly decreased again below 1% and the moulds regressed.

It is interesting to note that these two minor incidents, due to experimental problems, paradoxically confirmed the good working of the system.

TENTATIVE CONCLUSION : FURTHER DEVELOPMENTS EXPECTED

In seven months the silo will be emptied and the grain analysed from a microbiological and technological point of view. In particular the starch extraction qualities will be measured in pilot scale.

If all the results are good and if the state of plastic is also good, then a full scale trial (5000 m^3) will be organized, in collaboration with a starch making company. This will test the industrial feasability and the economic place for the system in the post-harvest handling structure existing in France.

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