# MICROBIOLOGICAL QUALITY OF GRAINS AND ERGOSTEROL CONTENT

**B. CAHAGNIER, Laurence LESAGE and D. RICHARD-MOLARD**

Institut National de la Recherche Agronomique
Laboratoire de Microbiologie et Technologie Céréalières
BP 527, 44026 Nantes Cédex 03, France

*SUMMARY*

The economic importance of moulds and the interest they arouse in terms of food technology and hygiene have been amply demonstrated. It is rather paradoxical to find that the analytical methods used to assess levels of contamination are flawed, especially in the case of solid products, inasmuch as the mycelial mass cannot be separated from the product. These methods are essentially based on measurement of sporulation of the species present, which results in long assays and hence delayed exploitation of the results (5 to 6 days after analysis).

A better approach consists in determining the fungal biomass formed. We determined this biomass by measurement of a specific cellular compound, ergosterol, in order to define the mycological quality of grains. We have shown the value of this compound by demonstrating a genuine independance between the growth conditions ($a_w$, oxygen levels) and the ergosterol content of the mycelia. The ergosterol assay involved solvent extraction, purification and HPLC. Analysis was rapid and a few hours were enough to obtain a result.

The ergosterol assay is also valuable in the complete absence of revivable microorganisms, since it is able to reveal past fungal infection. A survey performed over several years has led to definition of ergosterol levels commonly detected in good quality cereal grains at harvest. Threshold concentrations of ergosterol above which grains would be microbiologically spoiled have been defined during experimental storage of soft wheat, durum wheat, barley and maize.

*INTRODUCTION*

Ergosterol (ergostatriene 5, 7, 22 ol 3 β) is the major sterol in virtually all fungi, especially in the Ascomycotina and Deuteromycotina, and represents 90 % of the total sterols.

Ergosterol is principally a structural component of the cytoplasmic membrane of fungi, where it plays a major role and participates with phospholipids in regulation of transmembrane exchanges. It has also been isolated from mitochondria of moulds (**Barr** and **Heming**, 1972) where it appears to have an important role in the mitochondrial membrane (**Thompson** and **Parks**, 1972 ; **Elliot**, 1977). It would appear that there is a close relation between the presence of ergosterol and the respiration of fungi, in particular yeasts (**Parks**, 1978).

Cytoplasmic bodies are active sites in the apical zone of the hyphae which play a major role in the biosynthesis of the cell membrane of moulds, and which contain sterol esters as well as free sterols, notably ergosterol (**Parks**, 1978).

Sterols play an essentiel role in sexual reproduction, for example as precursors of sexual hormones such as antheridiol, the differentiation hormone of the spermatocysts of *Achlya bisexwalis* (**Barksdale**, 1969).

In numerous fungi, control of stimulation at spore germination and control of sporulation by conidia-forming hyphae, is in part dependent on certain sterols, including ergosterol (**Hendrix**, 1965 ; **Weete**, 1973).

Ergosterol is, therefore, a compound essential for the development, reproduction and life of a fungus. Its assay yields quantitative results in 2 to 3 hours. The presence of ergosterol in stored grains and seeds is a direct indicator of invasion by fungi, and moulds in particular. The specificity of this sterol is now sufficiently well established to justify the use of ergosterol assay for assessing the state of preservation of seeds and grains (**Seitz** *et al.*, 1977 ; **Cahagnier** and **Richard-Molard**, 1981 ; **Cahagnier** *et al.*, 1983 ; **Cahagnier**, 1984 ; **Naewbanis** *et al.*, 1986).

Results obtained in liquid medium at different water activities ($a_w$) indicate that the ergosterol content of mycelia is sufficiently constant, at least during the exponential growth phase (figure 1).

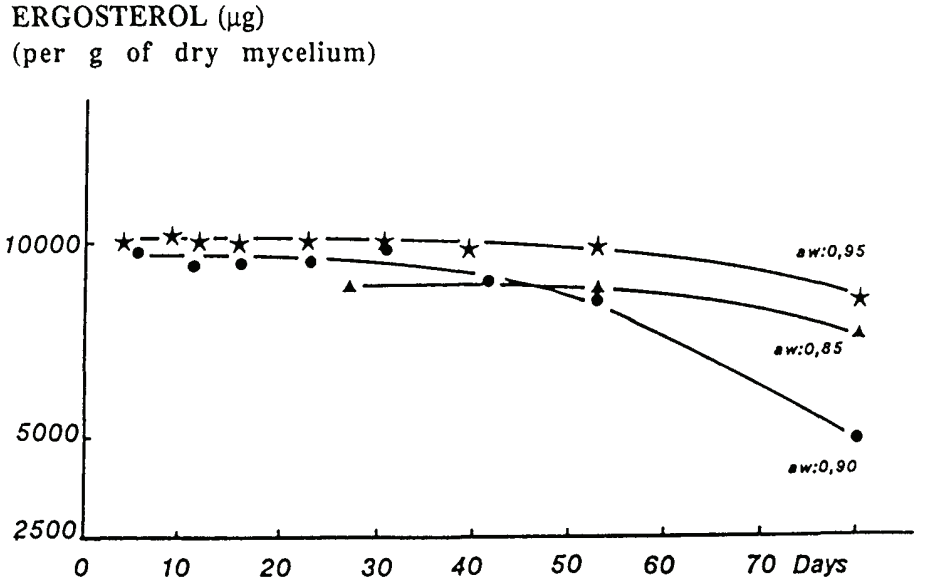



Figure 1 : Ergosterol content of *Aspergillus candidus* mycelium grown at various $a_W$ levels

Fungal spoilage can also be detected retrospectively using ergosterol assay, even when no dried, excised or simply old conidia are revivable. There is no possibility, however, of correlating ergosterol levels with mycotoxin content, whereas the correspondance is direct with fat acidity, a parameter also directly related to mould growth.

Currently, there are essentially two methods used to assess contamination of grains by moulds : the Ulster method or direct method which is applied to whole grains, and the conventional dilution-seeding method, which is also applicable to fractionated products. In the Ulster method (**Muskett** and **Malone**, 1956), the grains are placed on an agar nutrient medium in a Petri dish, either untreated in order to reveal external flora, or after surface disinfection in order to preserve only the thalluses in the outer tissues (internal flora). This method preserves the structure of the grains and may allow localisation of active colony-forming microflora visible on the grains. This method determines for a particular grain batch the percentage of grains contaminated by given genera or species of moulds, without however giving any indication of the degree of contamination. It also reveals poorly sporulating genera such as *Alternaria* and *Fusarium*, even in the presence of active spore-forming genera such as *Penicillium* and *Aspergillus*. Above a certain level of contamination, however, the direct method fails : contaminated grains can be distinguished from uncontaminated grains, but slightly and heavily contaminated grains cannot be differentiated.

In the case where seeds and grains are first disinfected, the degree of penetration of the disinfectant is unknown, and is a function of the nature and condition of the surface layers, which may vary from one assay to another, frequently rendering comparison of results hazardous.

The second type of commonly used method, and the only one presently standardised, involves enumeration of the fungal propagules on food products by suspension-dilution and visualisation on agar plates after 5 to 7 days of incubation.

In this type of analysis, often performed after grinding, thallus fragments are taken into account, but it is the fungal spores above all that are counted. The limits of such methods are soon reached though, since only revivable flora are taken into account, and dead microorganisms, which may testify to past fungal spoilage, remain undetectable. Furthermore, the assay is time-consuming and the results can only be put to use days after the analysis, which is naturally a major handicap, especially in the development of wet products by the food and agricultural industry.

Is should also be stressed that the extent of sporulation may vary considerably from one species to another, and for a given species will depend on growth conditions, and notably on the $a_w$ of the product. Now, the assay methods described essentially rely on sporulation as a criterion. Clearly, interpretation of the results in terms of product quality is problematical since the relations that may exist between sporulation rate and mycelium growth, the only true expression of fungal development are virtually unknown for solid products. The assay of ergosterol allows assessment of this biomass synthetized by moulds on a given substrate. The assay is valuable since the quantitative results are obtained quickly and the quantity of biomass thus estimated offers an improved indication of fungal activity for a substrate.

Over a period of years we have used this assay to define for different cereals the ergosterol content of a healthy grain, using also other criteria of quality such as fat acidity, germinative capacity and the number of fungal propagules. We have also defined acceptable limit values and threshold values above which fungal spoilage of products is considered to occur. These values were defined during storage tests on wheat, barley and maize at an $a_w$ of 0,90.

## *1. MATERIALS - EXPERIMENTAL PROTOCOL - METHODS OF ANALYSIS*

### *1.1. Materials*

The different batches of grains and seeds used (durum wheat, soft wheat, maize and barley) to define the ergosterol content of a healthy grain were harvested in 1981, 1982, 1984 and 1985 invarious parts of France (15 departments). The storage tests were performed on wheat, barley and maize grain from the Loire Atlantique department.

### *1.2. Experimental protocol*

The grains used to define the critical threshold were transferred to moisture conditions favourable to development of moulds. The storage tests were performed with batches of grain moistened by the addition of sterile water and then mixed at 5°C for 6 days, to give final moisture contents of 22% for barley, 20 % for wheat and 22 % for maize. The moistened grains were bagged in nylon mesh sacks, which were then suspended in 10-litre, non-airtight glass chambers. The volume of the grains represented about 1/5 of the volume of the chambers. The relative humidity of the atmosphere in the chambers was kept constant by establishment of equilibrium with a saturated aqueous solution of barium chloride. The 90 % relative humidity obtained was in moisture equilibrium with

the moistened grains. The temperature of the chambers was maintained at 24 ± 1°C.

### *1.3. Methods of analysis*

#### 1.3.1. Determination of grain moisture content

For all test grains, the moisture content of untreated grains was determined by oven-drying at 130°C of 10-gram samples for 38 to 40 hours in the case of maize, and for 15 hours in the cases of wheat and barley (AFNOR V-03-707). The results were expressed in % undried material.

#### 1.3.2. Enumeration of moulds

The fungal propagules present on the grains were counted after suspension in physiological solution according to AFNOR standard V-18-301 : 100 g of grain were poured under aseptic conditions into a 1-litre Waring-Blendor containing 400 ml of sterile diluent (8,5 g NaCl, 1 g Bacto-peptone Difco, 0,033 g Tween 80, distilled water to make 1000 ml, pH 7). After revival of the microorganisms by immersion for 20 minutes, the grains were ground for 1,5 min at a speed of 20 000 rpm. The starting suspension obtained in this way was subjected to microbiological analysis by the conventional dilution-method.

Fungi were detected using agar culture media, one containing 20 g of malt extract per litre, the other 50 g of malt extract plus 50 g of sodium chloride per litre, to both of which was added 0,1 g chloramphenicol per litre in order to eliminate development of bacterial colonies. The results were expressed as the number of propagules per gram of grain dry matter.

#### 1.3.3. Fat acitidy

The technique described in AFNOR standard V-03-712 was used to determine fat acidity, which essentially represents the free fatty acids extractable in 95 % (v/v) ethanol at room temperature. After centrifugation of the extract, an aliquot of the supernatant was titrated with 0,05 N sodium hydroxide, and the acidity was expressed conventionally in grams of sulphuric acid per 100 g of dry matter.

#### 1.3.4. Extraction and assay of ergosterol

The method used has been described previously (**Seitz** *et al.*, 1977 ; **Cahagnier** *et al.*, 1983 ; **Cahagnier**, 1984) and involved extraction and purification followed by HPLC assay. The main chromatographic conditions are indicated in figure 2, which shows a chromatogram of a maize extract.

FIGURE 2

Stainless steel column 125 x 5 mm

Stationary phase
Lichrosorb S160
Granule size 5 µ

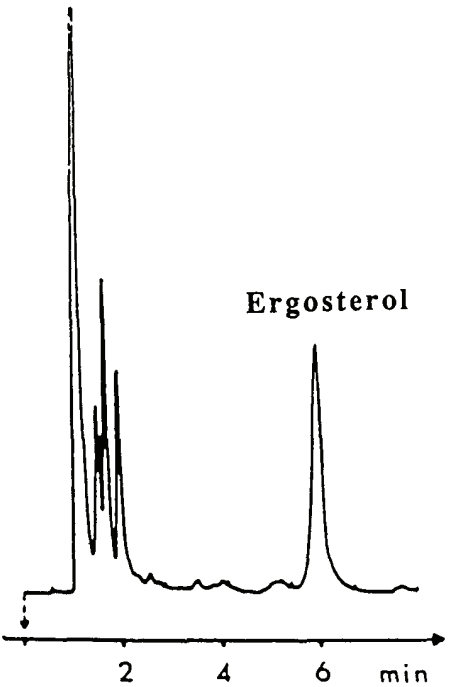
Mobile phase
methylene chloride
isopropanol (99.5/0.5, v/v)

Flow rate, 1.5 ml/min

Pressure, 450 P.S.I.

Volume injected, 10 µl

U.V. detection at 282 nm




### 1.3.5. Germinative capacity

The germinative capacity of the different grains studied was determined according to the standards of the International Seed Testing Association (ISTA), which has developed an internationally recognised series of tests. The germinative capacity is the percentage of grains capable of developing into seedlings considered normal in a defined optimal environment (temperature, moisture) within a fixed incubation time for each species.

## *2. RESULTS*

### *2.1. Ergosterol content of grains at harvest*

Before harvest, the grain carries so-called "field" mycoflora (Cahagnier, 1971), the composition and development of which depend greatly on the climatic conditions at harvest and on transport and handling operations before drying. The initial ergosterol content at the start of storage may vary from one year to another and from one grain to another. For soft wheat (table 1), ergosterol contents ranged between 2 and 4,8 µg/g dry matter, with a mean of 3,3 µg/g ($\sigma$ : 0,77). The number of moulds was low, not exceeding 900 propagules per gram. The

Ulster method revealed the presence of abundant *Alternaria* on the grains. The values of the technological criteria were wholly satisfactory : 99 % germinative capacity, and mean fat acidity of 0,0148 ($\sigma$ : 0,0014).

Hard wheat (table 2) gave ergosterol contents ranging from 3,2 to 6 µg/g, with a mean of 4,26 µg/g ($\sigma$ : 0,82). The number of propagules per gram was modest : 5,400 at most. Here too *Alternaria* was frequently present on may grains. The germinative capacity was 94 % and the mean fat acitity 0,016 ($\sigma$ : 0,0014).

In the case of barley (table 3), ergosterol contents ranged from 2,7 to 6,4 µg/g, with a mean of 4 µg/g ($\sigma$ : 1,70). The number of fungal propagules was small, the maximum count revealing 1,400 per gram, and as in the case of the wheats the barley grains carried many *Alternaria*. The technological values were good, since the germinative capacity was 97 % and the fat acidity 0,0235 ($\sigma$ : 0,0041).

For maize, the ergosterol contents ranged between 0,12 and 1 µg/g dry matter (table 4), with a mean of 0,45 µg/g. The number of fungal propagules ranged between 850 and 17 000 per gram, the dominant genus being *Cladosporium*. The mean values of the selected criteria were fully satisfactory: 99 % germinative capacity and fat acidity of 0,021 ($\sigma$ : 0,0025). These results reveal the distinct influences of the various genera of epiphytic mycoflora on grains and seeds. The genus *Alternaria* was highly dominant on the wheats and barley, causing relatively high initial ergosterol contents, particularly when the grains were harvested in a wet year (1981), as compared to a dry year (1982) (table 5). These results should be compared with the findings of **Seitz** *et al.* (1977) concerning the influence of a rainy period preceding the harvest and the presence of *Alternaria* (table 5). The low initial ergosterol levels in maize despite harvesting in France at high moisture contents (30 - 35 % undried material), suggest pronounced contamination by genera such as *Cladosporium*, *Epicoccum* or *Fusarium*. The ergosterol assay allows a much more quantitative analysis of grain-field mycoflora combinations, which is not possible with conventional enumeration methods because of the considerable variation in sporulation capacity among these fungi.

In light of the data listed in tables 1 to 4, it is clear that initial ergosterol contents vary substantially from one production to another, for climatic, agricultural and probably botanical (grain and seed anatomies) reasons. Despite the observed scatter in values, it seems possible to define the mean characteristic values for each grain and seed, the ranges being 0,2 to 1 µg per gram for maize.

### *2.2. Spoilage threshold*

In the tests on wheat, barley and maize, we have determined threshold values for ergosterol content above which fungal spoilage of the product can be said to have begun. The threshold values chosen for the other criteria were at least $10^5$ fungal propagules per gram of grain and/or fat acidity of 0,06, and a 20 % loss of germinative capacity.

At the start of storage, the flora enumerated on the grains consisted essentially of the genera *Alternaria* and *Fusarium* for the wheats and barley, and *Cladosporium* and *Epicoccum* for maize. After a few days of storage the mycoflora regressed in all cases and a more xerotolerant flora appeared, represented above all by *Aspergillus* (*candidus*, *niger*, *flavus*) and *Penicillium spp.*

TABLE 1 : Ergosterol content and fat acidity of moulds and germinative capacity of batches of soft wheat collected from different sources over two years.

| Source | Ergosterol g/g | | Moulds per g | | Fat Acidity | | Germinative capacity | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Aube | 2,3 | 4,3 | 39 | 60 | 0,014 | 0,016 | 100 | 100 |
| Aisne | 4,6 | | 900 | | 0,014 | | 100 | |
| Cher | 2,24 | 3,8 | 75 | 120 | 0,015 | 0,013 | 95 | 96 |
| Deux-Sèvres | | 3,8 | | 20 | | 0,016 | | 97 |
| Eure | 4 | 4,2 | 40 | 120 | 0,015 | 0,016 | 100 | 100 |
| Eure et Loire | 3,8 | | 500 | | 0,014 | | 100 | |
| Gers | 3,4 | 2,7 | 500 | 50 | 0,017 | 0,013 | 94 | 98 |
| Haute-Garonne | 3 | | 450 | | 0,014 | | 100 | |
| Loiret | 2,78 | 3 | 90 | 110 | 0,013 | 0,017 | 100 | 99 |
| Lot et Garonne | | 2,9 | | 450 | | 0,015 | | 96 |
| Marne | 2 | | 80 | | 0,013 | | 100 | |
| Nord | | 4,6 | | 100 | | 0,014 | | 99 |
| Oise | 3,1 | | 360 | | 0,015 | | 98 | |
| Somme | 3,8 | 3,2 | 80 | 30 | 0,018 | 0,014 | 100 | 96 |
| Sarthe | | 2 | | 90 | | 0,014 | | 96 |
| Seine-Maritime | | 3,9 | | 830 | | 0,015 | | 96 |
| Seine et-Marne | | 3 | 90 | 100 | 0,014 | 0,017 | 100 | 96 |

Table 2 : Ergostérol content and fat acidity of moulds and germinative capacity of batches of hard wheat collected from different sources over two years.

| Source | Ergosterol g/g | | Moulds per g | | Fat Acidity | | Germinative capacity | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Alpes | | 3 | | 190 | 0,011 | | | 99 |
| Aude | 5,4 | 5,1 | 4800 | 500 | 0,017 | 0,015 | 98 | 98 |
| Bouches du Rhône | 4 | 4,3 | 2400 | 420 | 0,015 | 0,019 | 90 | 96 |
| Cher | 6 | | 5400 | | | | | |
| Drôme | 4,8 | | 1800 | | | 0,016 | | 94 |
| Eure et Loire | 5,2 | 5 | 800 | 1200 | 0,015 | 0,015 | 90 | 93 |
| Gard | 4,8 | 4,9 | 4200 | 185 | 0,019 | 0,017 | 94 | 98 |
| Haute-Provence | 3,2 | | 84 | | 0,018 | | 92 | |
| Indre | 3,4 | 3,9 | 840 | 150 | 0,016 | 0,016 | 94 | 95 |
| Loiret | 3,8 | 4 | 240 | 50 | 0,016 | 0,02 | 93 | 92 |
| Loir et Cher | 4,7 | 4 | 500 | 160 | 0,014 | 0,014 | 90 | 91 |
| Vaucluse | | 4,6 | | 330 | | 0,018 | | 98 |

Table 3 : Ergosterol content and fat acidity of moulds and germinative capacity of batches of barley from collected different sources over two years.

| Source | Ergosterol g/g | | Moulds per g | | Fat Acidity | | Germinative capacity | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Allier | | 4 | | 220 | | 0,019 | | 96 |
| Ardennes | | 4,3 | | 53 | | 0,019 | | 96 |
| Aube | 7,4 | | 279 | | 0,025 | | 96 | |
| Cantal | | 3,8 | | 110 | | 0,021 | | 90 |
| Charente | | 4,16 | | 22 | | 0,025 | | 98 |
| Cher | 4,1 | | 130 | 22 | 0,02 | | 100 | |
| Charente-Maritime | | 4,3 | | 410 | | 0,02 | | 100 |
| Côte d'Or | 3,54 | 4 | 660 | 1400 | 0,023 | 0,019 | 96 | 96 |
| Dordogne | | 2,9 | | 20 | | 0,025 | | 99 |
| Eure | 3,6 | | | 80 | | 0,026 | | 90 |
| Eure et Loire | 3,1 | 3,6 | 98 | 41 | 0,026 | 0,027 | 100 | 94 |
| Gers | 4,96 | | 30 | | | 0,02 | | 98 |
| Indre | 8,4 | | 63 | | 0,023 | | 94 | |
| Indre et Loire | 6,4 | | 42 | | 0,025 | | 96 | |
| Loir et Cher | 5,1 | | | 200 | | 0,024 | | 97 |
| Marne | 4,3 | 4,4 | 21 | 240 | 0,023 | 0,02 | 100 | 97 |
| Meuse | 3 | 4,65 | 63 | 130 | 0,021 | 0,021 | 99 | 95 |
| Moselle | 2,72 | | 24 | | 0,036 | | 100 | |
| Nord | | 5 | | 94 | | 0,028 | | 99 |
| Oise | | 3,9 | | 25 | | 0,023 | | 98 |
| Seine et Marne | 4 | 3,2 | 90 | 40 | 0,027 | 0,023 | 100 | 99 |
| Yonne | 4 | 4,2 | 93 | 50 | 0,023 | 0,03 | 98 | 91 |

Table 4 : Ergosterol content and fat acidity of moulds and germinative capacity of batches of maize collected from different sources over two years.

| Source | Ergosterol g/g | | Moulds per g | | Fat Acidity | | Germinative capacity | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Allier | 0,9 | | 1700 | | 0,021 | | 99 | |
| Cher | 0,12 | 0,2 | 3400 | 2700 | 0,02 | 0,019 | 99 | 100 |
| Eure | | 0,9 | | 5200 | | 0,025 | | 99 |
| Eure et Loire | 0,36 | | 5200 | | 0,026 | | 100 | |
| Finistère | | 0,3 | | 2000 | | 0,02 | | 100 |
| Indre | 0,42 | 0,25 | 1000 | 1800 | 0,022 | | 100 | |
| Indre et Loire | 1 | | 1100 | | 0,025 | | 100 | |
| Loiret | 0,5 | 0,32 | 850 | 2800 | 0,025 | 0,02 | 100 | 99 |
| Loire Atlantique | 0,32 | 0,5 | 3800 | 9000 | 0,022 | 0,023 | 98 | 98 |
| Loir et Cher | 0,83 | 0,66 | 3800 | 9000 | 0,022 | 0,023 | 98 | 98 |
| Manche | 0,70 | | 8300 | | 0,019 | | 100 | |
| Marne | | 0,7 | | 3100 | | 0,02 | | 100 |
| Oise | 0,57 | 0,6 | 3000 | 4300 | 0,02 | 0,027 | 100 | 99 |
| Sarthe | | 0,46 | | 3400 | | 0,027 | | 98 |
| Seine et Marne | 0,37 | 0,4 | 8200 | 6000 | 0,023 | 0,022 | 100 | 99 |
| Seine et Oise | 0,5 | 0,43 | 3400 | 1900 | 0,02 | 0,024 | 100 | 99 |

Table 5 : Ergosterol content, fat acidity, dominant flora, and germinative capacity of batches of soft wheat from different sources.

| Source | Ergosterol µ/g | Moulds per g | % contaminated grains | Fat acidity | Germinative capacity (%) |
|---|---|---|---|---|---|
| Kansas (USA) | 0,67 | - | 9 *Alternaria* | - | - |
| Kansas (USA) | 1,7 | - | 16 *Alternaria* | - | - |
| Kansas (USA) | 3,9 | - | 90 *Alternaria* | - | - |
| Year 1981 Cher | 6 | - | 96 *Alternaria* 12 *Fusarium* | 0,016 | 99 |
| Haut-Rhin | 6 | - | 98 *Alternaria* 13 *Verticillium* | 0,015 | 100 |
| Indre | 6 | - | 92 *Alternaria* | 0,016 | 99 |

Table 6 : Ergosterol content and corresponding values of quality criteria

| Cereal | Ergosterol µg/g | Spores x $10^5$/g | Fat acidity | Germinative capacity | |
|---|---|---|---|---|---|
| | | | | Initial | after storage |
| Blé | 10 - 12 | 11 | 0,07 | 95 | 60 |
| Orge | 10 - 12 | 2 | 0,075 | 90 | 50 |
| Maïs | 6 - 8 | 1,5 | 0,11 | 94 | 75 |

According to the results obtained, the ergosterol threshold value was about 10 - 12 µg per gram for wheat and barley (table 6), and around 5 - 8 µg per gram for maize (table 6). The measured values of the other criteria equalled or exceeded the set thresholds.

Figure 3 illustrates curves of mould development and ergosterol content in batches of stored maize. These were used to determine the ergosterol content threshold value.

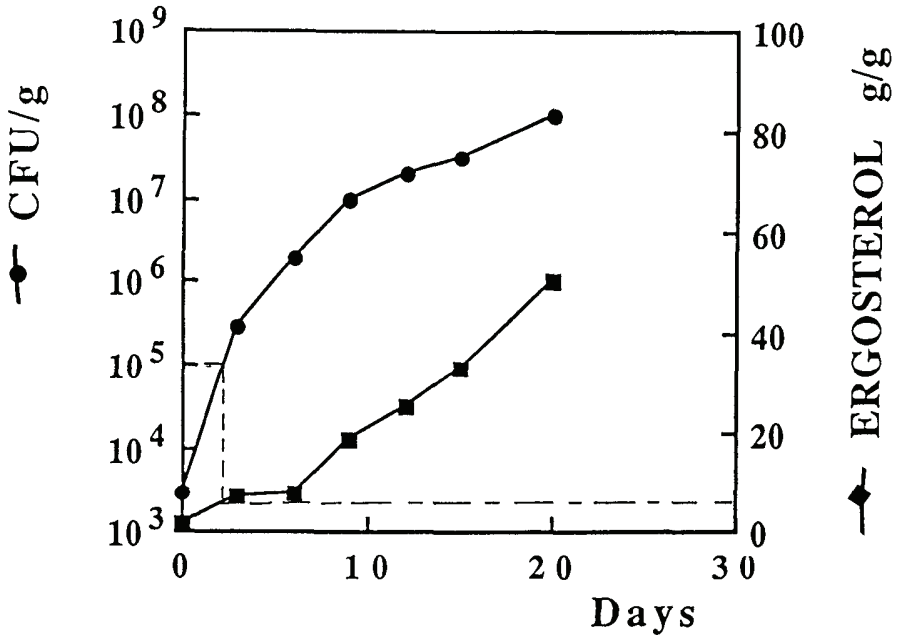



Figure 3 : Mould development and ergosterol content of batches of maize stored at 24°C and r.h. 90 %. Determination of the threshold value of ergosterol content

*DISCUSSION - CONCLUSIONS*

The nature of the field mycoflora established on the grain before harvest influences the initial ergosterol content, which may be high without signifying spoilage, as in the case of *Alternaria*-rich wheats, for example.

The initial ergosterol content was therefore higher in the case of *Alternaria*-rich wheats and barleys than in maize, in which it was virtually zero. The ergosterol content of the grains can be considered a better indicator of fungal invasion than criteria supplied by conventional analyses, which depend essentially on the sporulation of the moulds present. By contrast, enumeration

methods should be used if the aim is to detect the onset of mycofloral activity in insufficiently stabilised stocks, even if the results can only be put to use some days after the analysis. Indeed, as can be seen for the storage of maize (figure 3), the changes in the number of microorganisms per gram were always faster and larger than those in ergosterol content. Furthermore, qualitative analysis often allows detection of modifications in the dominant flora which are frequently revealing at the start of spoilage, even in the absence of increases in the total number of fungal propagules. However, when it is necessary to judge the technological or nutritional quality of ungraded grains, the best indications of the degree of spoilage are certainly given by analysis of mycelial growth. At the usual values of humidity and temperature, sporulation is, in fact, much less dependent on humidity than the ergosterol content.

It is clear that when the analysis reveals ergosterol contents in grain batches equal to or above the previously defined thresholds, the onset of fungal spoilage of the grains must be strongly suspected. In this case, it will be necessary to perform complementary analyses (identification of genera and species, of mycotoxins, tests to assess the technological value of the product) in order to verify the fungal invasion suggested by the ergosterol assay.

On the other hand, when the ergosterol content of a product is above thresholds of, for instance, 20 to 30 µg/g, then there can be no doubt that the grains are highly contaminated by moulds, as the laboratory (**Cahagnier**, 1984, 1986) and field results amply demonstrate.

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# QUALITE MICROBIOLOGIQUE DES GRAINS ET TENEUR EN ERGOSTEROL

**B. CAHAGNIER, Laurence LESAGE et D. RICHARD-MOLARD**

Institut National de la Recherche Agronomique
Laboratoire de Microbiologie et Technologie Céréalières
BP 527, 44026 Nantes Cedex 03, France

## RESUME

L'importance économique des moisissures et l'intérêt qu'elles suscitent au plan de la technologie et de l'hygiène alimentaires ne sont plus à démontrer. Il est quelque peu paradoxal de constater que les méthodes d'analyse qui permettent d'apprécier les niveaux de contamination sont très imparfaites, surtout pour les produits solides, dans la mesure où la masse mycélienne ne peut être séparée du produit. Ces méthodes s'appuient essentiellement sur la mesure de la sporulation des espèces présentes qui entraîne des manipulations longues, donc une exploitation tardive des résultats (5 à 6 jours après l'analyse). Une meilleure approche consiste à déterminer la biomasse fongique formée. C'est vers une détermination de cette biomasse par mesure d'un composé spécifique lié à la particule cellulaire, **l'ergostérol**, que nous nous sommes orientés pour définir la qualité mycologique des grains. Nous avons montré l'intérêt de ce composé en mettant en évidence une réelle indépendance entre les conditions de croissance ($a_w$, taux d'oxygène) et les taux d'ergostérol des mycéliums. Le dosage lui-même s'effectue par HPLC après extraction par solvant et purification. L'analyse est rapide et quelques heures suffisent pour obtenir un résultat.

On peut noter également l'intérêt du dosage d'ergostérol a posteriori qui prouve une infection fongique passée même lorsque tout germe revivifiable a disparu. Une enquête qui a porté sur plusieurs années, a permis de définir les teneurs en ergostérol habituellement détectées dans des céréales de bonne qualité à la récolte. Des valeurs seuils d'ergostérol au-delà desquelles les grains seraient microbiologiqument altérés, ont été définies au cours de stockages expérimentaux pour les blés tendres et durs, l'orge et le maïs.