Effect of water-activity on mold growth and mycotoxin production

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EFFECT OF WATER ACTIVITY ON MOLD GROWTH AND MYCOTOXIN PRODUCTION

D. Richard-Molard, L. Lesage and B. Cahagnier

1. INTRODUCTION

Molds which contaminate agricultural products, foods and feeds, form a very heterogenous group of microorganisms in respect of their water requirements. Most microfungi occurring in such products need very high a_w, close to the maximum, to develop, but some genera include species able to grow at much lower a_w levels. Jarvis (32) makes the distinction between hygrophilic fungi, unable to develop below 0,90 a_w, mesoxerophilic species which can grow at a_w 0,90 - 0,80 and xerophilic fungi capable of growth below a_w 0,80. Pitt (65) defines xerophiles as "fungi which are capable of growth, under at least one set of environmental conditions, at a_w below 0,85". This limit seems to be of some practical interest but it is probably better today to consider such species as "xerotolerant" (11, 14, 21) and to reserve the term "xerophilic" for the very few molds which are markedly inhibited by high a_w levels like *Monascus bisporus*.

Among about 5 000 genera of fungi now recognized, only eleven contain xerotolerant and xerophilic species ; almost all of these species are Ascomycetes or probable asexual forms of them (65). Adaptation to reduced a_w, which allows the growth of extreme xerophiles down to 0,62 - 0,65 a_w, represents therefore a mechanism restricted to a very few highly specialized species.

Compared to many hygrophilic microfungi, xerotolerant molds, which belong mainly to genera *Aspergillus*, *Eurotium* and *Penicillium*, are also characterised by a very high ability to sporulate. Thus they have a great ability to disseminate which probably explains the high frequency of contamination in the agricultural and food industries and more particularly in products with medium or low moisture content,

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in the range of 0,90 - 0,60 a_w. On the other hand, true xerophiles are often reluctant to sporulate so that some very important xerophilic species are not universally found.

Long believed to be harmless, molds, or at least some of them, are now known to produce molecules highly toxic for man and animals. Intensive research over thirty years in the field of mycotoxins has shown that about 150 - 200 species of microfungi are potentially toxigenic (32, 52). Mycotoxigenic fungi include some hygrophilic strains like *Fusarium* species but most of them are capable of growth below 0,90 a_w and this connexion between xerotolerant and mycotoxigenic fungi gives the true dimension of the problem of mold growth in foodstuffs. Fortunately, few of the "real" xerophiles are toxigenic and there are few reports of mycotoxin production below 0,85 a_w.

Although a variety of molds may grow on the surface of foods of animal origin such as dry sausages or some dairy products (9, 39, 61), the best substrates for mold growth and mycotoxin production have been shown to be stored seeds, their products and further processed foods. Examples given in the present paper will be taken in this field.

2. INFLUENCE OF A_W LEVELS ON MOLD DISTRIBUTION IN FOOD MATERIALS

Xerotolerant molds occur everywhere. The primary inoculum for most of them is found on decaying plant material and agricultural products of plant origin are always but generally scantily contamined in the field. After the harvest, grains, seeds and other low a_w ecological niches are frequently recontamined by a secondary inoculum of xerotolerant and xerophilic molds present in elevators, mills or factories (64). In many processes, primary contaminants are inactivated by heating and mold growth on bakery goods or dried fruits is due to recontamination by this secondary inoculum.

Due to this constant contamination, food spoilage by molds depends mainly on environmental factors, the water activity being doubtless the most important to be considered (65, 74, 79). Foodstuffs with water activities above 0,90 - 0,93 are generally more subject to rapid bacterial spoilage than to fungal spoilage (Fig. 1). This is not because fungi cannot grow at so high a_w, but because in such conditions bacteria are highly competitive, growing faster than yeasts and molds (10, 80). Spoilage by bacteria, including pathogenic species, represents the major factor limiting the storage life of so called "highly perishable products", such as fish and meat. Nevertheless, moulding is often a problem in this a_w range with fresh fruits or recontaminated bakery goods. Below 0,90 to 0,85 a_w, only some bacteria (cocci, lactic bacteria) can still grow and spoilage

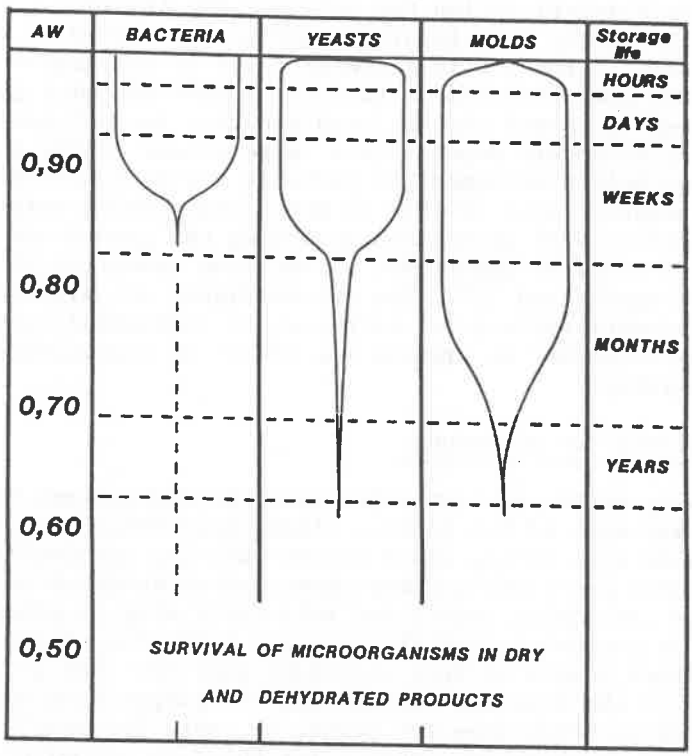

Figure 1. Diagram of microbiological spoilage of foodstuffs in relation to water activity.

by yeasts and molds becomes predominant although occuring more slowly.

Below 0,85 a_w, all non-halophilic bacteria are inhibited and in particular *Staphylococcus aureus* (42), which is certainly the most xerotolerant pathogenic bacterium contaminating foods. In this a_w range, xerophilic (or osmophilic) yeasts are still able to grow or to slowly develop fermentative activities in products with high sugar or salt contents such as jams, dried fruits, honey, dry sausages and hams. As a general rule, yeasts prefer liquid or semi-liquid media probably due to their relative "immobility" and tolerance to anaerobiosis (85). On the other hand, and owing to their mycelial growth, molds appear to be microorganisms best adapted to solid substrates with low moisture contents such as grains and their products.

3. WATER ACTIVITY, MOLD GROWTH AND MYCOTOXIN PRODUCTION

Biochemical, technological and nutritional consequences of microfungi in grains and related products are well known today. They include such damages as decrease in germinative capacity, dry matter

losses, lipid deterioration and self-heating in silos (20, 73). At present, one of the most important questions related to mold growth in foodstuffs is the possible biosynthesis of mycotoxins and most of the research now conducted in this field concerns this question. Nevertheless it should not be forgotten that in most cases the attitude of consumers depends to a large extent on the visual aspect and obvious mold development is probably the main factor in refusing finished products even if they do not contain toxic metabolites. So the understanding of mechanisms governing the growth and sporulation of molds is of first importance in the food industry. In addition, it must be emphasized (55) that the available detoxification methods are still unsatisfactory for technical or economical reasons, so that it remains necessary to prevent the growth of xerotolerant molds in food materials.

3.1. Life cycle of microfungi

Basically different from unicellular microorganisms such as bacteria and most of the yeasts, filamentous fungi have a more or less complex life cycle, which begins with the germination of a dormant spore producing a first hypha called promycelium. Except under very particular conditions this first step is generally followed by extension, ramification and formation of cross-walls in hyphae. After a more or less important pure mycelial growth, depending on the external conditions, the fungus develops more differentiated structures for sexual or, more frequently, asexual reproduction as shown in Figure 2 for *Eurotium chevalieri*.

This species can reproduce either asexually (*Aspergillus* form with conidiophores producing phialospores) or sexually with ascopores formation. Each of these main developmental phases is characterized by different water requirements ; germination occurs at lower a_w than

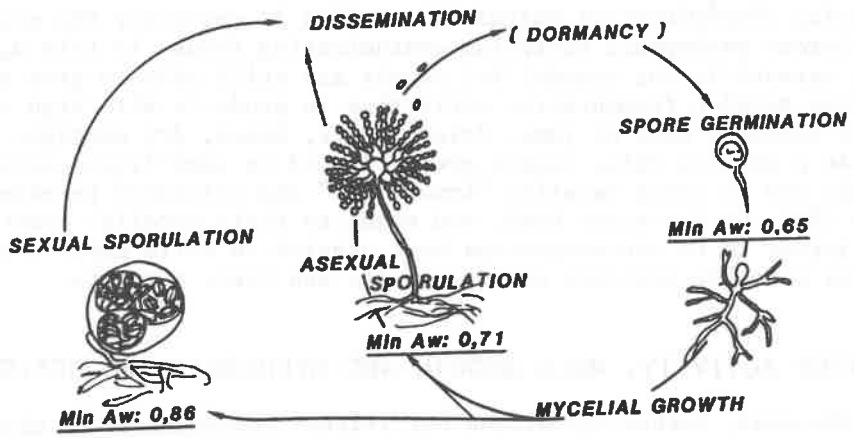

Figure 2. Life cycle of Eurotium chevalieri and a_w requirements.

asexual sporulation and sexual reproduction requires higher a_w levels (57, 66, 83). For practical purposes, limiting a_w levels for sporulation are the most relevant, since they allow fungi to complete their life cycle, which is the condition for extensive growth and marked degradation of substrates (65).

3.2. Limiting a_w levels allowing mold growth

As shown in Figure 3 (86), in liquid culture medium, the mycelial growth of molds follows the classical growth curve of microorganisms. It begins with a lag phase followed by a phase of rapid growth which can be compared to the exponential growth of unicellular microorganisms. After the maximum stationary phase, a phase of decline can be observed due to progressive autolysis of aged mycelium. Schematically, as recalled by Troller (87), reducing a_w levels of the substrate increases the lag period, decreases the rate of growth and reduces the maximum level of development (Fig. 4). At the limit, all other conditions being optimal, the lag phase can be considered as unlimited and the minimum a_w value permitting the growth of a given species is so established.

Since the introduction of the water activity concept in food microbiology (54, 78, 79), the a_w limiting levels for numerous molds, especially xerotolerant and xerophilic species, have been published. Recently reviewed by Pitt (65) and Beuchat (11), some of them are listed in Table I in which are included values published by Snow (83), Pelhate (65), Pitt and Christian (66), Ayerst (3), Mislivec and Tuite

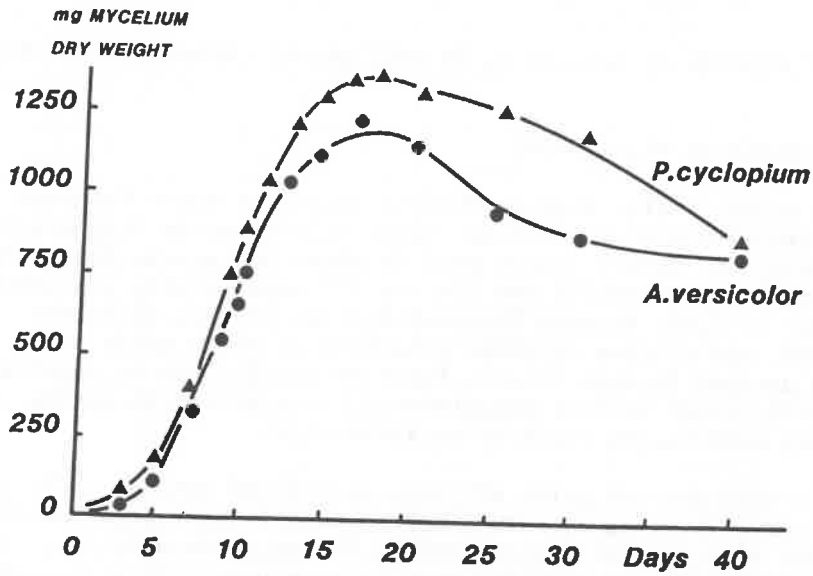

Figure 3. Growth curves of Penicillium cyclopium and Aspergillus versicolor (stationary cultures on liquid medium).

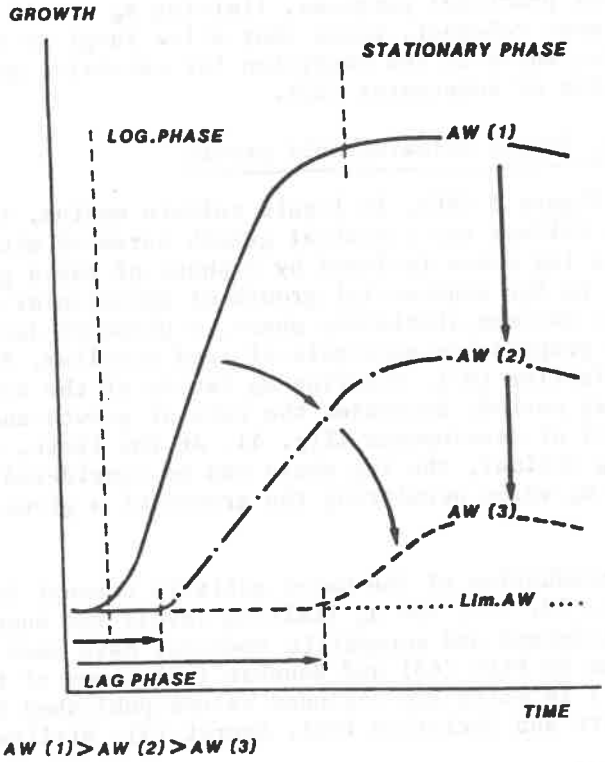

Figure 4. Effects of reduced a_w on mold growth - Adapted from (87).

(50), Northolt et al. (56-58).

Quite surprisingly, data previously published about *Fusarium* species are very scarce, although these species occur very frequently on at least some cereal grains such as wheat and maïze. According to Schneider (77) and Armolik and Dickson (2) some species can develop at a_w near to 0,90. Because *Fusarium* species produce different mycotoxins, new studies on water relations of these molds are urgently needed. To some extent, *Fusarium* species can be considered as parasitic fungi so that experimental inoculations on grains in laboratory experiments could be questionnable.

From a more general point of view, as pointed out by Seiler (80), most of these limiting a_w values were obtained under experimental conditions with optimal culture media. It now seems necessary to complete them with observations made in more practical situations taking into account for instance the biochemical composition of the substrate or the interaction which can occur between strains. This

Table I. Approximate minimum a_w for growth of some molds at temperature near optimal.

Fungal species	a_w	Reference
Stachybotris atra	0,94	(31)
Botrytis cinerea	0,92	(83)
Trichothecium roseum	0,90	(63)
Penicillium funiculusum	0,90	(50)
Fusarium sp	0,90	(2)
Cladosporium herbarum	0,88	(83)
Aspergillus wentii	0,84	(66)
Penicillium cyclopium	0,85	(63)
Penicillium islandicum	0,83	(3)
Penicillium expansum	0,82	(29)
Aspergillus flavus	0,80	(56)
Aspergillus niger	0,77	(3)
Aspergillus ochraceus	0,77	(66)
Wallemia sebi	0,75	(63)
Eurotium chevalieri	0,71	(3)
Eurotium amstelodami	0,70	(2)
Chrysosporium fastidium	0,69	(66)
Eurotium echinulatum	0,65	(63)
Monascus (Xeromyces) *bisporus*	0,61	(66)

point of view leads to experiments in which the growth of fungi is more carefully related to the expected shelf-life of feeds and foods stored under real conditions of time, temperature or packaging (19, 55, 62, 76). So in the coming years, one can expect the setting up of "practical" a_w levels for stability with respect to mold growth, at least for some important groups of foods or raw materials, taking into account the actual storage times in industrial conditions.

3.3. Water activity and mycotoxin production

Mycotoxins are nonantigenic substances, toxic for man and animals, which can be elaborated by molds in various foods and feeds but especially in cereal grains, other seeds and their products. Some of these toxic fungal metabolites, such as aflatoxin B_1, are carcinogenic, the others producing various disease syndromes, so that every effort must be made to prevent food contamination by mycotoxins. Mycotoxigenic fungi are principally found in three genera : *Fusarium*, *Aspergillus* and *Penicillium* (30, 52, 65). *Fusarium* species are known to be hygrophilic but most of the other are more or less xerotolerant.

Some of the limiting a_w values for mycotoxin production are listed in Table II, in which data recently reviewed by Leistner et al. (41) and Northolt and Bullerman (55) are complemented mainly by those of Northolt et al. (56-58), Lotzsch and Trapper (43, 44), Le Bars (37,

Table II. Limiting a_w levels for mycotoxin production (minimum reported values).

Mycotoxins	Fungal species	a_w	Reference
Aflatoxin	*Aspergillus flavus*	0,80	(13)
		0,83	(56)
		0,82	(43)
	Aspergillus parasiticus	0,87	(56)
Penicillic acid	*Aspergillus ochraceus*	0,80	(5)
		0,88	(57)
	Penicillium cyclopium	0,97	(57)
Ochratoxin A	*Aspergillus ochraceus*	0,85	(5)
		0,83	(57)
	Penicillium cyclopium	0,87	(57)
	Penicillium viridicatum	0,83	(43)
Patulin	*Penicillium sp*	0,88	(11)
Cyclopiazonic acid	*Penicillium sp*	0,87	(11)
Roquefortine	*Penicillium sp*	0,87	(11)
Citrinin	*Penicillium sp*	0,90	(11)
PR Toxin	*Penicillium sp*	0,90	(11)
Penitrem A	*Penicillium sp*	0,94	(11)
Zearalenone	*Fusarium sp*	-	-
Trichothecenes	*Fusarium sp*	-	-
Moniliformin	*Fusarium sp*	-	-
Sterigmatocystin	*Aspergillus sp*	-	-
Islanditoxin	*Penicillium sp*	-	-
Luteoskyrin	*Penicillium sp*	-	-
Rubratoxin	-	-	-

38) for others mycotoxins such as ochratoxin A, patulin, penicillic and cyclopiazonic acids. Data on the a_w levels required for mycotoxin formation are still scarce, as shown in Table II, and important differences are often evident among published values for one given species producing a given mycotoxin. This is probably due to the nature and composition of the growth substrate which seems to have a great influence on metabolic pathways leading to mycotoxin accumulation.

At present, most available data deal with aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus* on various agricultural products and large differences exist in the limiting a_w values so far published

for aflatoxins. The lowest a_w levels permitting aflatoxin B_1 biosynthesis are near to 0,80 (13) but limiting values of 0,83 - 0,85 a_w are frequently reported. Penicillic acid is also produced at very low a_w levels (0,80) as shown by Bacon et al. (5) in a study with poultry feeds inoculated with *Aspergillus ochraceus*. With both *Penicillium viridicatum* and A. *ochraceus*, ochratoxin A begins to accumulate in laboratory media at 0,83 a_w (57).

As a general rule, it is considered that the limits of toxin production are higher than those for growth, but this introduces the very important question of the influence of mold growth itself on the a_w of the substrate : it is indeed well known that due to respiration, heat and metabolic water are produced during growth so that the activity of water is locally and progressively increased (20, 53, 72). This is why the concept of limiting a_w levels for mycotoxin production must be used with caution, especially for products such as grains and seeds which can be stored for long periods.

It seems that for processed foods and feeds such an evolution presents only a limited risk because, according to Jarvis (32), contamination by mycotoxins never occurs before an extensive growth of molds so that alterations become visible or at least perceivable by sensory evaluation. Nevertheless, the situation can be quite different with raw materials for which subsequent drying or other treatments could mask fungal deterioration without destroying the toxins. One of the most acute problems in this regard is probably sneak contaminations of animal feeds with aflatoxin B_1, leading to aflatoxin M_1 in milk. As indicated by Leistner et al. (41), limiting a_w levels for toxin production are also dependant on the levels at which toxins can be detected. Thus, new developments in methods for toxin detection could make it necessary to verify some old data and to determine more accurately the minimal conditions of time and temperature for biosynthesis in products themselves, even for the well known aflatoxins.

The very low toxicity of xerotolerant species in the *Eurotium glaucus* group is well established (12), but information on the conditions required for toxin production by *Fusarium spp* is still very scarce. Results obtained by Sherwood and Peberdy (82) suggest limiting a_w close to 0,90 for zearalenone production : significant concentrations ($\geq$ 100 ppm) have been obtained after 10 months on cereal grains with 23 % moisture content (0,92 - 0,93 a_w) but because of the experimental conditions employed, these results remain difficult to interpret. With barley, data from Enari et al. (25) confirm the possibility of toxin formation during storage (wet grains stored in cribs for instance) near or even below 0,90 a_w, but only if contamination of the grain by *Fusarium sp* occurs before harvest. As far as we know, nothing has been yet published for trichothecenes or moniliformin.

4. ADAPTATION TO REDUCED A_W

Studies on the evolution of conidial water content, in *Penicillium roqueforti* for instance (7), demonstrate an active dehydration of mold spores by syneresis during their formation and maturation. The production of conidia with water contents lower than those of the initial mycelium seems to be a general phenomenon in molds and could be considered a first step in adaptation to reduced a_w. A concomitant process is the accumulation of polyalcohols in the cytoplasm (6), but until recently these solutes were considered merely energy storage substances. After their dissemination, fungal spores remain dormant due to specific water soluble inhibitors, as shown by Krishnan et al. (34), in *Aspergillus niger*. As soon as sufficient water becomes available, the repressive effect of the inhibitor disappears by dilution and germination can occur. Such mechanisms are not yet well known but they could explain the great differences that are observed in water requirements for fungal spore germination.

On the other hand, it is clear that the growth of microorganisms at reduced a_w is possible only if the cells are able to accumulate solutes to balance the osmotic pressure on the membrane. In bacteria, ions and amino-acids play this role of "compatible solutes" (27, 49). In yeasts, the compatible solutes seem to be polyalcohols, mainly glycerol and arabitol (15-17) but other results suggest more complex mechanisms for osmoregulation (1, 51). In fungi, recent work confirms the role of glycerol as an osmoregulatory substance (28, 46, 47) and shows that mechanisms involving potassium ion accumulation also occur.

The intracellular accumulation of osmoregulatory substances can be achieved either by absorption or by synthesis (24). If because of the substrate composition, the cell is obliged to synthesize such solutes, it utilises energy. As a general rule, one can perhaps consider that the lower is the outlay of energy for osmoregulation, the higher is the tolerance to reduced water activities.

5. INTERACTIONS BETWEEN A_W AND OTHER PARAMETERS

Basically, with systems at equilibrium, thermodynamic water activity of substrates is doubtless the most influential parameter. Nevertheless, other parameters and especially temperature, chemical composition and physical structure of substrates, or gazeous environment, may have a considerable influence on mold growth.

5.1. Particular effects of temperature

Beside the generally well known effect of temperature which produces an increase in water requirements as soon as the system is shifted from the optimal temperature for mold growth, more particular effects can be observed. In recent experiments (Fig. 5) we have shown

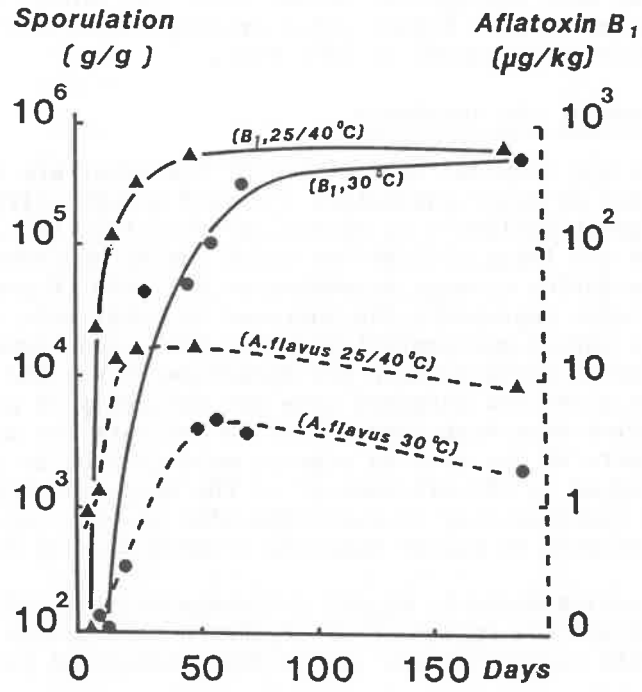

Figure 5. Sporulation and mycotoxin production by Aspergillus flavus on rice (a_w : 0,93) (—) Aflatoxin production at (●) 30°C. (---) Sporulation under (▲) cycling temperature 25-40°C.

an activation of growth and metabolic activity by cycling temperatures. Compared to a constant equivalent temperature of 30°C, cycling temperatures varying between 25°C and 40°C, i.e. about 10 hours at 25°C followed by 10 hours at 40°C, lead to an increased sporulation rate for *Aspergillus flavus* on rice and a faster aflatoxin synthesis (84).

In these experiments simulating the thermal variations which can be undergone by grain during storage, relative humidity is "controlled" by a salt solution ($KH_2 PO_4$) giving 92 % RH at 30°C and oscillating between 91 % RH at 40°C and 93 % RH at 25°C (89). So probably only slight variations of a_w are introduced in the system by such cycling temperatures and the mechanism of activation is not yet clear. It could be due to an increase in the availability of water at the surface of the grain where molds are located, but another possibility is a particular response of fungi to such conditions which could be studied by calorimetry. This effect has been confirmed with maïze at various relative humidities (Richard-Molard and Cahagnier, 1983, unpublished results) and similar results have

been obtained with *Aspergillus parasiticus*, the biosynthesis of aflatoxin B_1 being even higher under cycling temperature (5/25°C) that a constant temperature of 25°C (60).

5.2. Influence of the substrate

Structure and chemical composition of the substrate are generally not considered as major parameters for mold growth, although they may have a great influence on mycotoxin production (55). In Figure 6, are shown the large differences which can be observed in mycoflora growth rates during storage depending on the kind of grains and seeds (70). Each curve represents the increase in total mold count with time. From a purely mycological point of view, such comparisons cannot be considered as valid because the mycoflora is not the same in each case but for practical purposes they are obviously of great interest. Similar studies have been carried out to estimate the ability of the different parts of the seed to support mold growth. As a general rule, the higher is the oil content of the seed, the more rapid the growth. The accessibility of nutrients also plays a very important role, as indicated by faster moulding of mechanically damaged grains.

Recent results shown in Figure 7 (Lesage et al., 1983, unpublished) demonstrate the influence of lipids on mold growth at reduced a_w. The growth of *Penicillium implicatum*, inoculated from a pure

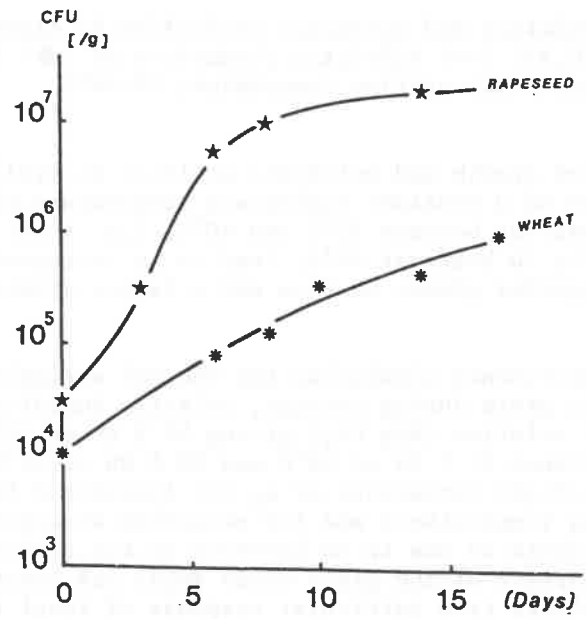

Figure 6. Influence of the substrate on the growth speed of total mycoflora (E.R.H. 85 %. Temperature 22°C) Adapted from (70).

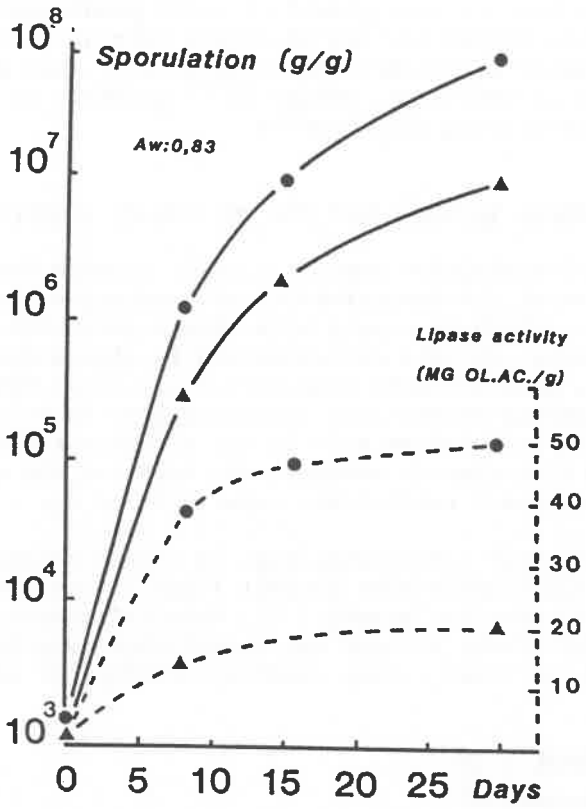

Figure 7. Influence of neutral lipids on (—) sporulation and (---) lipase activity of Penicillium implicatum on (●) whole and (▲) defatted meal of maïze (lipase activity is expressed as fatty acids produced from triolein by one gramm of moldy meal.

culture, is faster on whole coarse meal of maïze than on defatted meal, neutral lipids being previously removed by Soxhlet extraction. This effect was observed at 0,83 a_w, well above the limiting a_w level for the growth of *Penicillium implicatum* (0,78 a_w) (29), and could be much more important nearer to this limit, suggesting some connexion between the lipolytic ability of molds and their xerotolerance.

Lipids are also implicated in aflatoxin biosynthesis and correlations have been demonstrated between the lipolytic activity and the capacity to produce mycotoxins in *Aspergillus flavus* toxigenic strains (4, 26, 36). For *Aspergillus parasiticus*, toxinogenesis is stimulated by saturated fatty acid (myristic, palmitic and stearic acids) whereas unsaturated fatty acids seem to have inhibitory effects (71).

These few examples show the influence that the temperature or the substrate may have on mold growth or toxin production and as a consequence the impracticality of taking into account only the activity of water to predict the shelf-life of food products, in the present state of knowledge, unless it is possible to reduce a_w below the minimum permitting mold growth.

6. MOLD GROWTH DETERMINATION ON SOLID SUBSTRATES

Mold growth estimation remains a major problem for solid foodstuffs because of the impossibility of separating the mycelium from the substrate, which is simple with liquid substrates of synthetic laboratory media. So this determination is always made by indirect measurements, more often by enumeration of colony forming units, such units representing essentially asexual spores in xerotolerant fungi. Unfortunately, sporulation rate is not a constant criterion and varies greatly from a species to another, and even for one species depends on the environmental conditions, especially on a_w.

Under particular conditions, e.g. if oxygen becomes the limiting factor, the sporulation rate in most fungi is considerably reduced as compared to mycelial growth (75). Mycelial growth and toxinogenesis of *Aspergillus flavus* without any sporulation have been observed on cheeses (61) and other recent studies confirm that aflatoxin formation

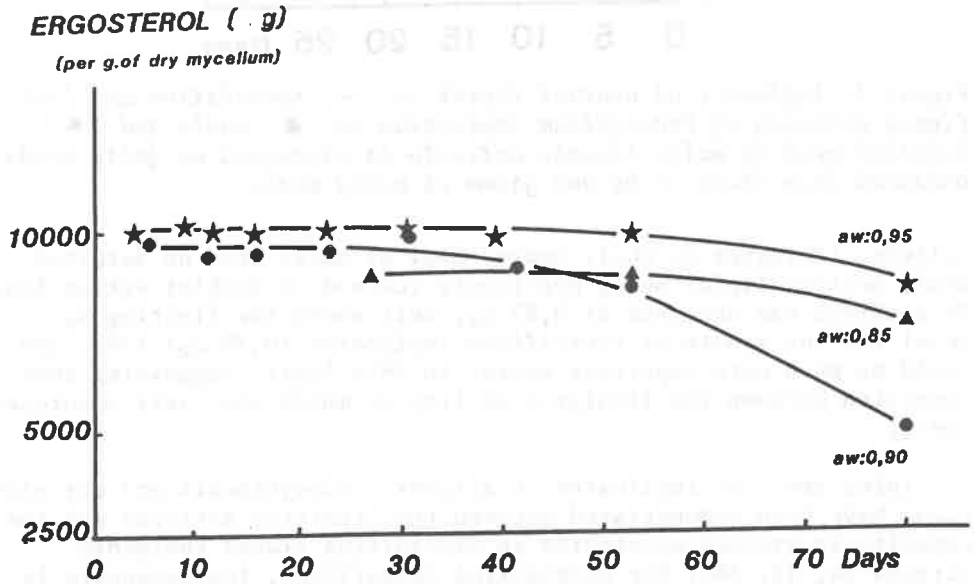

Figure 8. Ergosterol content of Aspergillus candidus mycelium grown at various a_w levels.

is not necessary correlated with sporulation (8). This obviously represents an important problem for both public health and methodology.

Different techniques have been proposed or effectively used to overcome this problem. Landers et al. (36) measured the accumulation of fatty acids in peanuts invaded by *Aspergillus flavus*. But this criterion depends both on the substrate and the species and cannot be generalised. The determination of chitin, a cell wall component for most of fungi has been used with success on various foodstuffs (22, 23). For grains and seeds frequently invaded by insects and mites, such determinations can lead to major errors.

Another possibility is the measurement of ergosterol content (81). This mycosterol found in fungal membranes is quite specific of fungal growth in the grains so far studied (18). Recent results (Fig. 8) obtained in liquid synthetic media with *Aspergillus candidus* show that the ergosterol content of mycelium grown at various a_w can be considered constant at least during the "exponential" growth. Taking experimental error into account, it can be claimed that the ergosterol content of mycelium varies only slightly with a_w in the range 0,80 - 0,95 (Richard-Molard and Cahagnier, 1983, unpublished).

In contrast, Figure 9 demonstrates the absence of a linear relationship between sporulation and total mold growth estimated by ergosterol determination. If a_w is reduced from 0,93 to 0,85, sporulation curves show only a slight retardation, whereas ergosterol content demonstrates that actual fungal growth is reduced at least three-fold. These preliminary results indicate that such a method gives a more objective estimation of mold growth in solid media. This could be of use in the study of growth and toxin formation by *Fusarium* species which do not sporulate readily.

7. CONCLUSIONS

Some aspects related to water requirements of microorganisms in foods have been neglected in this short review devoted to fungi, when they seemed to be of little practical interest. The kind of solutes added for instance in intermediate moisture foods to reduce a_w, which is of great importance for bacteria, seems to affect fungal growth only very slightly (67). Likewise, molds are relatively indifferent to the pH of foods or raw materials in the range usually encountered.

The effect of a_w on viability and survival of fungal spores is not basically different from that on bacterial spores and the heat resistance of fungi, as of bacteria, increases at reduced a_w (23, 48). Molds are also more susceptible to water soluble antifungal agents such as propionic acid or to gamma-irradiation at high a_w levels (68, 69). Obviously the complete destruction of fungi in foods

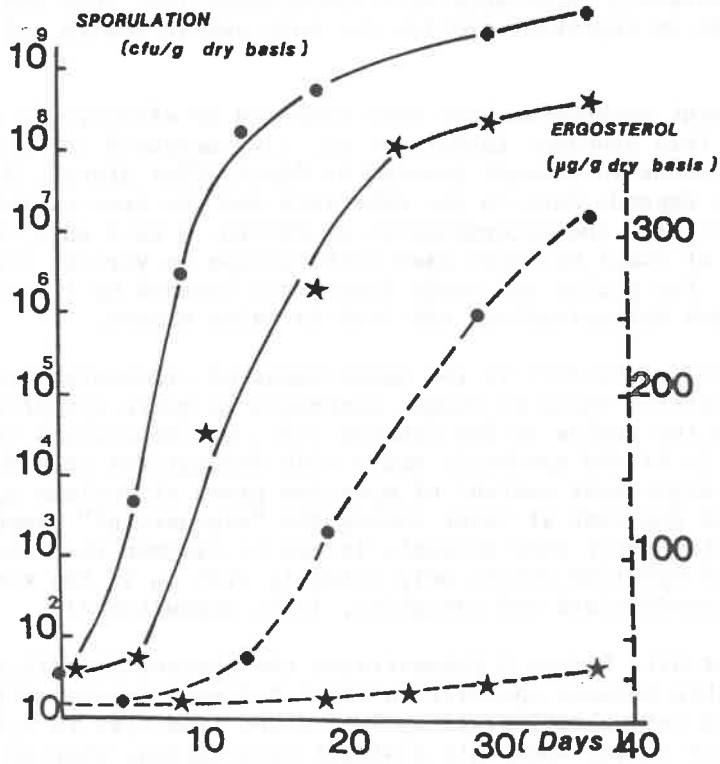

Figure 9. Comparison of sporulation and mycelial growth (estimated by ergosterol content) of Aspergillus candidus on maize at (●) 0,90 a_w and (★) 0,82 a_w.

is an impossible objective in the present rate of food technology. In most cases, the treatments which should be necessary remain incompatible with technological and organoleptical qualities, even when economically possible. Even for baked products with sterile surfaces such as bread, problems of moulding exist due to recontamination after baking. Thus, the primary aim in food preservation against xerotolerant molds is to prevent or to adequately retard their growth and metabolic activity. As said before, because of consumer preferences, the preventing growth and sporulation appears to be as important for food technologist as the prevention of mycotoxin formation.

If sufficient time is available, xerotolerant fungi are certainly the most capable microorganisms to overcome the various "hurdles" (40) which can be usually utilised : reducing a_w, cooling and freezing, airtight packaging or addition of antifungal agents like propionates or sorbates if processes or legislation allow such addi-

tions. For economic, technological or organoleptical reasons, it is often impossible to set up these hurdles at levels that control xerotolerant molds, and further quantitative research is needed, in practical situations, on the effects of several hurdles in combination on the moulding of naturally contaminated foods.

From the toxicological point of view, very little is yet known about possible synergisms between several mycotoxins simultaneously produced on the same commodity. For example, severe nephrotoxicosis due to a probable synergy between ochratoxin A and citrinin has been reported by Krogh et al. (35), whereas Vesela et al. (88) claimed a simple additive effect of these two mycotoxins. With cereal grains invaded by *Fusarium* species, several toxic substances are often produced together and basic information on the resulting toxicity is urgently needed.

On a more fundamental level, much remains to be done to fully explain the mechanisms of adaptation to reduced a_w in microfungi. In particular, it would be very interesting to understand how xerotolerant and xerophilic molds perceive variations of water activity, as such knowledge could lead to new methods for preventing fungal growth.

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