INFLUENCE OF MAIZE LIPIDS ON THE GROWTH AND LIPOLYTIC ACTIVITY OF *EUROTIUM CHEVALIERI* AT REDUCED WATER ACTIVITIES

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ABSTRACT

At reduced water activities, *Eurotium chevalieri* Mangin, a xerotolerant mold, was shown to grow more or less markedly according to the lipid content present in maize millings. A higher growth estimated by ergosterol content was found in the lipidic medium. The availibility of water to microorganisms was quite similar in media whatever the lipid content as shown by sorption isotherms ; it cannot be involved in higher growth found in the lipidic medium. A fungal lipase activity was detected and shown to be responsable of decrease in maize lipids (triglycerides) ; unsaturated fatty acids were preferentially released from lipid hydrolysis.
In the adverse conditions of strongly reduced a_w, free fatty acids released from maize lipid hydrolysis could be the sole available carbon source allowing fungal growth.

INTRODUCTION

In general, most xerotolerant fungi which invade cereals during storage belong mainly to the genera *Penicillium.*, *Aspergillus* or *Eurotium* (Pitt, 1975 ; Richard-Molard *et al.*, 1985) This adaptation to growth on solid substrates with low moisture contents is not well understood. Fungal infection leads to an increase in the amount of free fatty acids (Hutchinson, 1961 ; Lesage *et al.*, 1985) and to a change in chemical composition of stored grain (Wallace *et al.*, 1983). As shown by sorption isotherms (Guilbot and Lindenberg, 1960 ; Poisson and

Cahagnier, 1979) a higher concentration in lipids leads to a higher availibility of water to microorganisms. In practical situations, lipid hydrolysis always takes place more rapidly than degradation of other components in the stored grain (Christensen and Kaufmann, 1965). For this reason, the acid value in infected grains is commonly used as a sensitive index of deterioration during storage (Drapron and Berger, 1976 ; Farag *et al.*, 1985). Moreover, lipolytic activity was shown to occur at low water activities, where other enzymatic activities become extremely weak (Acker and Beutler, 1965 ; Caillat and Drapron, 1970).
This report gives information about the relationship between the degradation of cereal lipids and the ability of xerotolerant molds to grow at reduced a_W.

MATERIAL AND METHODS

Mold species. The microorganism used in this study was the xerotolerant fungus, *Eurotium chevalieri* Mangin (holomorph form described in Thom and Church, (1926)) isolated from maize. The cultures were cultivated on wet maize grains to allow inoculation.

Culture conditions. French maize grains with 5.2 % total lipids (on a dry basis) were ground (0.4mm in diameter) and sterilized for 40 min at 110°C. This milling was the full-fat medium (FM). Defatted medium (DM) was obtained from FM n-hexane extraction according to Genot *et al.* (1984). The two media were divided among many 250 mL conical flasks (12 g of medium a flask) and adjusted to convenient a_W 0.84 and 0.74 by adding sterile water. Actual a_W values were determined with an electric hygrometer (Rotronic, Zürich, Switzerland). Inoculation of 10^4 colony forming units (CFU) per g was done by placing a glass bead (3 mm) covered with *E. chevalieri* fungal propagules into each conical flask of FM and DM. After shaking for 10 min, the glass beads were removed. This inoculation procedure did not modify the a_W of the medium. Cultures were incubated at 30°C in non-hermetically sealed containers in which a constant relative humidity, 84 % and 74 % RH, was maintained by use of saturated potassium chloride and sodium nitrate solutions, respectively (Bizot *et al.*, 1978). Samples were taken, in duplicate, at intervals according to the growth rate of *E. chevalieri* . Experiments were repeated at least once.

Sorption isotherms. Adsorption isotherms of both millings, FM and DM, were established following the procedure of Spiess and Woll (1983). The curves were directly plotted using the mathematical model of Guggenheim-Anderson-De-Boer (Bizot, 1983).

Fungal growth . Fungal growth was estimated by plate count and by measurement of ergosterol content. All the samples analyzed were at least in duplicate. For the dilution plating method, to samples (2 to 4 g) were added 100 mL diluent (0.85 % (w/v) NaCl ; 0.1 % (w/v) Bactopeptone

Difco ; 0.0033 % (w/v) Tween 80 ; pH = 7.0) and vigorously shaken for 20 min. Malt extract agar containing 0.01 % (w/v) chloramphenicol and supplemented with 5 % (w/v) sucrose - 7 % (w/v) NaCl was used as test medium according to Pitt (1975). Plates were incubated for one week at 25°C. The results were expressed in CFU/g of dry milling.
Ergosterol content was extracted from samples by the method of Seitz *et al.* (1977) modified by Cahagnier *et al.* (1983). High performance liquid chromatography (HPLC) analysis was carried out with a LDC Constametric II G pump system and a Merck Lichrocart cartridge (125 mm x 4 mm) packed with μ Lichrosorb SI 60 (5 μm) coupled to a LDC Spectromonitor III variable wavelengh detector set at 282 nm. The mobile phase was methylene chloride-isopropanol (99.5 : 0.5 ; V/V) at 1.5 ml/min. Ergosterol content was expressed as μg/g dry milling. Uninoculated maize milling samples were used as controls.

Lipid analyses. Free lipids were extracted from FM with n-hexane by the method of Genot *et al.* (1984). The free lipid extract, dried under nitrogen and weighed, was redissolved in n-hexane containing heptadecanoic acid ($C_{17:0}$) as internal standard. Free fatty acids were separated from FM free lipid extract as described in Lesage *et al.* (1985) and esterified by the diazomethane technique (Schlenk and Gellerman, 1960) before analysis by gas liquid chromatography (GLC). A Delsi 300 gas chromatograph equipped with a flame ionization detector and a capillary column (70 m length : 0.35 mm internal diameter) coated with Carbowax 20 M were used with oven temperature of 180°C, and detector and injector temperature of 200°C. The flow rate of helium used as a carrier gas was 20 ml/min with a split ratio of 1/40. Quantitative FFA determination was made by comparison with ($C_{17:0}$) as internal standard.

Lipase Activity . Fungal lipase activity was measured from defatted millings, DM and defatted residual product from FM n-hexane extraction, with the procedure of Drapron and Sclafani (1969). Free fatty acid estimation was made by CPG as previously described. Lipase activity was expressed in an arbitrary unit, i.e. μ moles $C_{18:1}$/g dry defatted milling/72h.

RESULTS .

Growth of *E. chevalieri* was monitored by ergosterol and colony forming units assays (Figure 1).

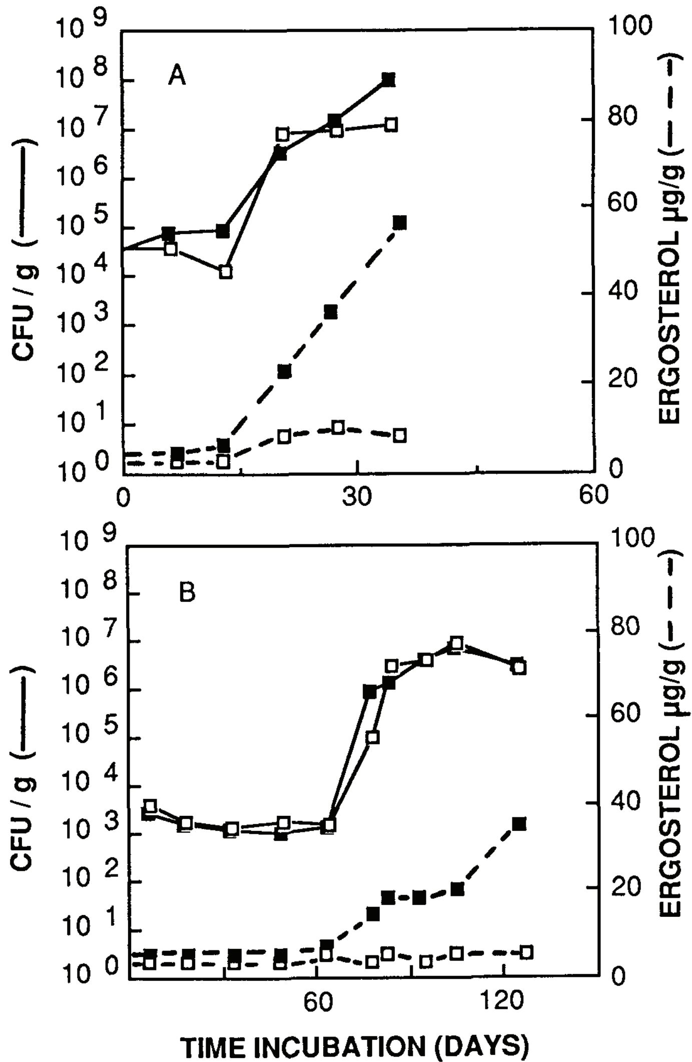

Figure 1 : Growth of *E. chevalieri* at a_w 0.84 (A) and 0.74 (B). Number of CFU/g and ergosterol content in FM (■) and DM (□).

At a_w 0.84, the lag phase did not exceed 13 days whereas it was at least 60 days at a_w 0.74. After that period, the propagule formation (CFU/g) was very active. An increase from 10^4 to 10^8 and 10^7 CFU/g at a_w 0.84 and 0.74, respectively, was showed at approximatively the same rate in both fatted (FM) and defatted (DM) media. Over the same time period,

ergosterol content increased to reach 55 and 35 µg/g of dry matter in FM compared to 7 and 5 µg/g in DM, respectively at a_w 0.84 and 0.74.

Adsorption isotherms of FM and DM were showed in Figure 2.

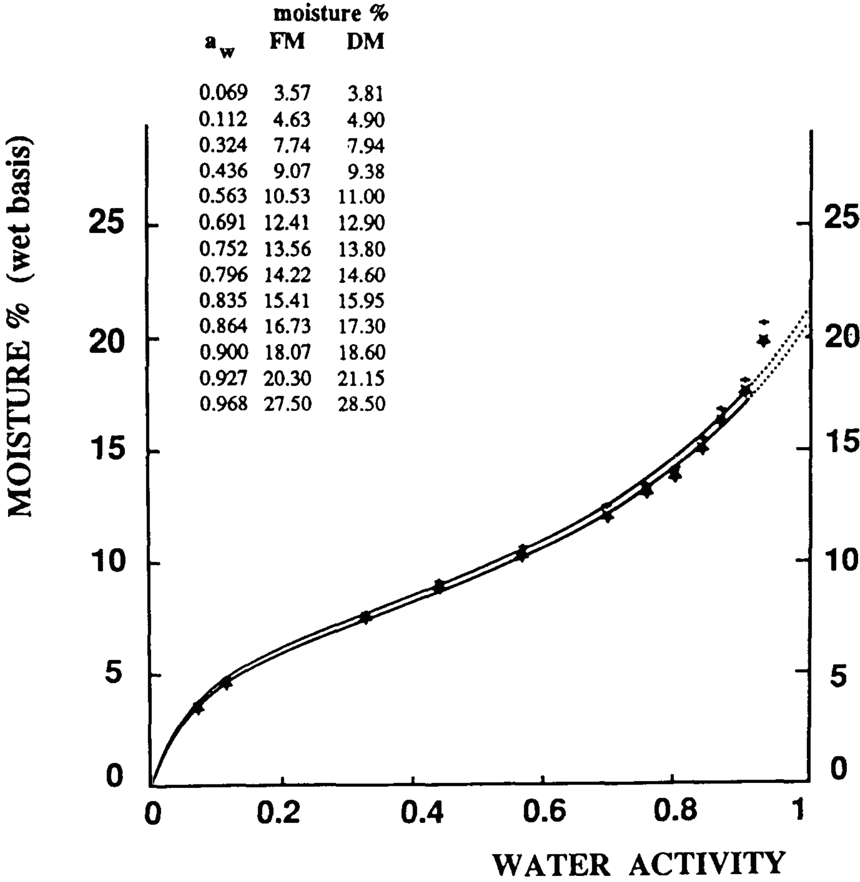

Figure 2. Adsorption isotherms of FM (lower curve) and DM (upper curve) according to Guggenheim-Anderson-De Boer model (G.A.B). Moisture content in percent on a wet basis.

Only a very slight increase in moisture content was produced by lipid removal at water activities between 0.60 and 0.90.

As shown in Figure 3, the level in free lipids of FM incubared at a_w 0.74 decreased gradually from 4.30 to 1.95 % (dry weight basis) whereas the free fatty acid values increased markedly from 0.34 to 1.8 % to decrease gradually after 20 days until the end of the experiment. While fungal lipase activity was detectable in fatted and defatted millings, it was greater in the fatted milling (Figure 3). The concentration of individual fatty acids was characterized by an increase in oleic and linoleic acids which corresponded to a higher level of unsaturation (Table I).

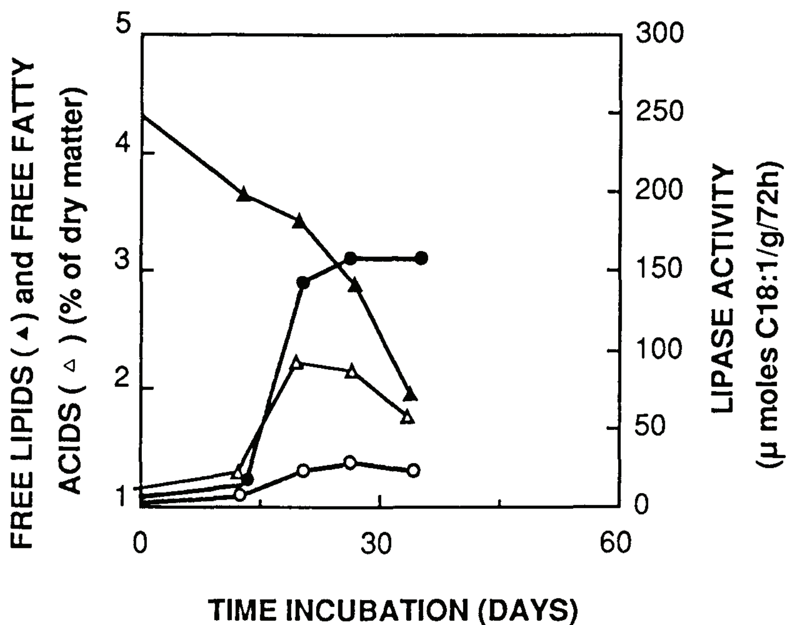

Figure 3 : Maize lipid metabolism of *E. chevalieri* at a_w 0.84. Free lipid (▲) and free fatty acid (△) contents from FM and lipase activity in FM (●) and DM (o).

Table I. Free fatty acid distribution of fatted medium inoculated with *E. chevalieri* and stored at a_W 0.84 and 30°C.

Fatty acids[2]		Storage period (days)				
		Blank[1]	13	20	27	34
palmitic	$C_{16:0}$	17.4	13.5	8.3	6.3	5.1
stearic	$C_{18:0}$	3.4	2.6	-	-	-
oleic	$C_{18:1}$	27.8	28.1	34.4	34.9	36.0
linoleic	$C_{18:2}$	49.3	53.8	56.2	57.8	58.2
linolenic	C18:3	2.1	2.0	1.1	1.0	0.7
Δ/mole [3]		1.33	1.42	1.50	1.53	1.54

1. Free fatty acids of sterilized maïze milling
2. Recorded as percent of the sum of the free fatty acids (average of four values)
3. Values for unsaturation of fatty acids (Δ/mole) were calculated as (% monoene + 2 % diene + 3 % triene)/100

DISCUSSION AND CONCLUSIONS.

A xerotolerant strain, *Eurotium chevalieri*, was studied in order to investigate the influence of lipid content of cereal medium on their ability to grow, especially at reduced a_w, such as 0.84.

After a short lag phase, the propagule formation was very active (Figure 1) and it was not possible to distinguish between the growth on fatted medium (FM) and defatted medium (DM). On the contrary, considering the growth curves established on the basis of ergosterol determination it was obvious that fungal growth was slower on DM. As shown by Cahagnier *et al.* (1983) and Miller *et al.* (1983), the ergosterol content was more sensitive as indicator of relative fungal biomass than the viable counts. By the way, preliminary experiments with liquid synthetic media, described by Cahagnier (1984), supplemented with various percentage of maize oil have established that higher intrinsec sterol production in mycelia obtained on high fat millings was not responsible of increased total ergosterol contents (data not shown).

Similar adsorption isotherms of DM and FM (Figure 2) at 30°C assumed that water availability to microorganisms did not contribute to the higher fungal growth level found in FM compared to that of DM, in contrast to results expected (Poisson and Cahagnier, 1979).

In addition to growth studies, maize lipid metabolism was followed. Lipase activity of *E. chevalieri*, still active at low water activity (Guilbot, 1967 ; Acker, 1969), increased with growth and was enhanced when lipids were present in the medium (Figure 3). Lipolytic enzymes were stimulated by lipids. Some authors have reported similar stimulations of microbial lipases by their substrates in other growth conditions (Ruban *et al.*, 1978 ; Sviridenko *et al.*, 1978 ; Ogundero, 1980). Free fatty acids were produced progressively from lipid hydrolysis by fungal lipase during initial growth (Figure 3) and then were assimilated by *E. chevalieri* as shown by their progressive decrease. Modifications in free fatty acid distribution were observed (Table I). Fungal lipase removed especially oleic and linoleic acids. This enzymatic activity exhibited strong specificity for long and unsaturated fatty acid chains. Further investigations would be necessary. Antonian (1988) gived recent advances in the characterization of fungal lipases.

Many authors described cereal lipid deterioration during storage by xerotolerant fungi but a few tried to understand more about use of these lipids for fungi growth. With *E. chevalieri*, a common cereal xerotolerant fungus, we showed that at low water activity, the ability for a mold to grow is clearly related to the lipid composition of the medium.

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TENEUR EN LIPIDES ET DEVELOPPEMENT DES MOISISSURES DES STOCKS DE MAIS PAR EUROTIUM CHEVALIERI AUX FAIBLES ACTIVITES DE L'EAU

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RESUME

On montre qu'aux activités de l'eau réduites, la croissance d'*Eurotium chevalieri* est dépendante de la teneur en lipides des grains. Une plus forte croissance, estimée d'après le contenu en ergostérol, a été trouvée en milieu lipidique. On a détecté une lipase fongique active qui s'est avérée être responsable d'une réduction des lipides du maîs (triglycérides).

A l'inverse, avec une a_W réduite (au-dessous de 0,80), la libération d'acides gras libres par hydrolyse des lipides du maîs pourrait être la seule source de carbone disponible qui permettrait la croissance fongique.