CHAPTER 4.0 THE INCIDENCE OF FUNGI IN STARCH-BASED FOOD STORED AT DIFFERENT LEVELS OF WATER ACTIVITY AND THEIR ROLE IN BIODETERIORATION

4.1 INTRODUCTION

Fungal colonization of foods originated from plants begins in the field by field fungi and rarely develop further in store. Examples of predominant field fungi on cereals are species of *Alternaria, Cladosporium* and *Fusarium*. Storage fungi are seldom detected before harvest but more inoculum is added during harvesting, drying, transport and storage. Storage fungi predominantly belongs to the genera *Aspergillus* and *Penicillium* which are adapted to low moisture conditions. However, *A. flavus* species which is considered as storage fungi in temperate climate may be of significance in the field in the warm climate (Hill *et al.*, 1985; Lacey, 1986). Invasion by fungi before harvest is governed primarily by specific commodity-fungus relations, while invasion by postharvest fungi is governed primarily by physical and chemical environmental factors.

Fungi may be found in stored cereals as inoculum on the surface of grains as superficially adhering propagules (such as spores, sclerotia and conidia) or concomitant contaminants in the form of infected plant, soil or animal debris mixed up with the substrate. The microflora of stored products is determined by the microbial inoculum it carries and the conditions of its storage (Magan and Lacey, 1984a,b,c). Studies of fungal incidence in foodstuffs in Southern Africa (Keen and Martin, 1971; Martin, Gilman and Keen, 1971) have shown that the species composition of each foodstuff is often characteristic.

The attributes which permit fungi to act as opportunist spoilage organism include the ability to grow at reduced A_W levels, within the range 0.65 (for extreme xerophiles) to 0.90 and above e.g. fungi from the genus *Aspergillus* (Christensen, 1978); grow at reduced pH values (to pH 3 and below); grow over a wide range of temperature i.e. from <0 °C to >40 °C; utilize a wide range of substrates; sporulate freely in a variety of conditions thereby extending contamination within a particular habitat.

Quantitative estimation of fungi occurring on stored food must always be followed by their identification. Only this allow us to indicate the conditions in which it has been stored and therefore help undertake proper measures to choose appropriate methods to protect stored foodstuffs against microorganisms and to reduce losses. So far, no ideal universal method for determination of fungi on foods or feeds has been developed (Jarvis *et al.*, 1983). The choice of a particular method will very much depend on the type of investigations. The most widely used technique for enumeration of fungi on food is direct plating and dilution plate method. A wider range of species may be enumerated by using different media and incubation temperatures. Included in this study is a media with low A_w i.e 7.5% sodium chloride-added malt extract agar to obtain realistic estimates of xerophiles.

It has been shown that the development of fungi on various materials is conditioned by the equilibrium relative humidity of the storage atmosphere (Lacey, Hill and Edwards, 1980) rather than by the moisture content of the materials themselves. Identification of the species causing damage is essential in order to reduce the humidity of the place of storage to a safe level at which spore germination cannot take place. Few species can germinate at low relative humidity (Gottlieb, 1978). Snow (1949), observed that young germ tubes require a higher humidity for their subsequent active healthy growth than is essential for the early stages of germination from the dormant spore. Where this is not provided misshapen chlamydospore structures are produced by the germ tubes. Optimum growth of most field and storage fungi occurred close to 1.00 A_w. The only exceptions were *A. amstelodami*, *A. repens* and *A. versicolor* with optima between 0.90 and 0.95 A_w (Magan and Lacey, 1984a).

Several workers have investigated various aspects of the problem of deterioration of rice (e.g. Schroeder and Sorenson, 1961; Christensen and Lopez, 1965; Fanse and Christensen, 1966; Lopez and Christensen, 1967; Christensen, 1969; Jayaraman and Kalyanasundaram, 1994), barley grains (Hill and Lacey, 1983) and wheat grains (Magan and Lacey, 1984a,b,c) but relatively little information is available on the conditions that determine the prevalence of individual kinds of these fungi on glutinous rice and flours.

Many storage fungi are toxin producers, hence, an accurate identification of toxigenic fungi in the mycoflora of stored commodities under known environmental condition is very important in the analysis of mycotoxins present for the monitoring of mycotoxin contamination in food. To be able to restrict the number and the complexicity of mycotoxin analysis, it is important to determine the connection between fungal taxa and profiles of mycotoxins (Frisvad, 1986; Svendsen and Frisvad, 1994).

The earlier survey on mycoflora count (Chapter 2) showed that starch-based food sold in the market in Malaysia usually harbours abundant storage fungi. The inconspicuous nature of the fungi could facilitate the consumption of deteriorated starch-based food, especially by the less discriminating consumers. Hence, this study is undertaken to identify the fungal species capable of proliferating in the course of short-term storage at different levels of water activity and to determine their roles in biodeterioration of starch-based food.

4.2 MATERIALS AND METHODS

4.2.1 Fungal incidence on starch-based food stored at different levels of water activity

4.2.1.1 Incubation of starch-based food

The effect of A_w on the occurrence of fungal species on starch-based food were determined by exposing 200g of each composite samples prepared from Method 3.2.1 (of Chapter 3) to atmospheres of known equilibrium relative humidities for the equilibration of substrate to a water activity ranging from 0.65 - 0.98 as described in Appendix B. At time intervals of 3, 7, 12, 19, 26, 33, 54, 75 and 96 days samples were taken for fungal analysis using direct plating and dilution plating methods on three types of media as described below.

4.2.1.2 Direct plating method

This method was employed to enumerate fungi which are actually invading the food. Grains were washed with sterile distilled water and dried on filter paper. Five grams sample were then plated onto three types of agar plates. Five gram samples of flours were directly plated onto agar plates.

4.2.1.3 Dilution plating method

To determine superficial contaminants adhering on grains or contaminants from foreign sources mixed up with the grains, 10 mls of sterile distilled water was added to 5g of ordinary rice or glutinous rice. This was shaken thoroughly and 1 ml of the contaminated water was pipetted into sterile petri dishes. For flour samples, 5 g were homogenized in a sterile blender with 10 mls of sterile distilled water for 2 mins. Immediately after blending, 1 ml of the suspension was pipetted using wide-tipped pipettes into sterile plastic petri dishes.

Nine millimeters of molten cooled agar was then poured into the petri dishes and swirled lightly. These dilutions gave at least 20 fungal colonies per plate.

4.2.1.4 Media used for the enumeration of fungi

The enumeration of fungi using each method was carried out on three types of media to allow for variations of fungal species. One replicate of each media was used for each method. Czapek-Dox agar (CDA) and potato dextrose agar (PDA) was used to enumerate most fungi especially of the *Aspergillus* and *Penicillium* species. 7.5% sodium chloride-added malt extract agar (MEA) was used to encourage growth of xerophilic species. The composition of each media was given in Appendix A.

To prevent bacterial growth, each medium were supplemented with 100 mg/l chloramphenicol before autoclaving.

4.2.1.5 Incubation and analysis of plates

All inoculated plates were incubated at 25 °C in stacks of not more than five. Each plate were examined for growth of fungi every 24 hrs until 10 days. The colonies of slow growing fungi were transferred to a different plate for identification. Species of *Aspergillus* were identified according to Raper and Fennell (1965) while species of *Penicillium* were identified according to Ramirez

(1982). Difficult species were sent to The Commonwealth Mycological Institute (CMI) for confirmation. Others were identified only to the genus.

4.2.2 Participatory roles of isolates in biodeterioration of starch-based food

The roles of fungi in biodeterioration were obtained by determining the rate of growth of fungi, amylolytic activity of fungi and the fungal induced changes i.e. pH and reducing sugar released after 7 days of fungal growth in starch-extract media as described in Appendix A. Fungi used in these studies represent the dominant and important species isolated from starch-based food stored at different levels of A_w and maintained on Czapek-Dox agar slants. Fungi tested were *Aspergillus flavus*, *A. niger*, *A. candidus*, *A. terreus*, *A. fumigatus*, *A. clavatus*, *Penicillium chrysogenum*, *P. islandicum*, *Rhizopus arrhizus*, *R. microsporus*, *Absidia corymbifera* and *Moniliella* sp.

For each fungal species, six replicate Petri dishes containing starch-extract agar were inoculated centrally, each with a 5-mm agar disc of mycelium (4-day old) and incubated at 25 °C for 7 days. Colony diameters were recorded every day until either the colony touched the edge of the plate or over a period of 7 days. Growth rates in mm per day diameter were calculated during the linear phase of growth. The amylolytic activity was determined using the method of the Society of American Bacteriologists (Society of American Biologists, 1957). Three replicate plates were flooded with an iodine solution (KI, 15g; I_2, 3g per litre of distilled water). A zone void of blue indicating the region of amylolytic activity was measured. For the remaining three plates, the entire contents were poured into clean mortars and a pestle was used to crushed and mix the contents. The amount of soluble reducing sugar released in one millilitre of the contents was estimated as maltose by the modified Somogyi-Nelson method (Miller, 1959; Appendix B). Standard curves were prepared from the spectrophotometric readings using known quantities of standard aqueous maltose. Ten millilitre of distilled water was added to the remaining contents and mixed before determining the pH.

Similar determinations using contents of plates inoculated with blank agar disc served as the control.

4.3. RESULTS

The frequency of occurrence of fungi on six starch-based food incubated at seven different levels of A_W ranging from 0.65 - 0.98 for 96 days are presented in Appendix D(4.1) and summarized in Tables 4.2 - 4.7. Two methods of plating and three types of media were used for each method i.e. a total of six replicates per sample were made. Hence, the occurrence of fungal species on $\geq$ 5 plates were designated as very frequent (V), 3 or 4 plates as moderately frequent (M) and $\leq$ 2 plates as rare (R).

These profiles were compared with the fungal profile at day 0 also presented in Appendix D(4.1). Five replicates of each starch-based food were analysed. Hence, the total number of plates made per substrate were 30 and fungal species occurring on > 20 plates were designated as very frequent (V), 11 - 20 plates as moderately frequent (M) and $\leq$ 10 as rare (R). Table 4.1 presents a summary of the incidence of fungi present on composite samples of each starch-

Table 4.1 The incidence of fungi on composite samples of starch-based food before storage (Day 0) at different levels of water activity

STARCH-BASED FOOD FUNGAL SPECIES	Ordinary Rice	Glutinous Rice	Riceflour	Glutinous Riceflour	Wheatflour	Cornflour
***Aspergillus* species**						
A. aculeatus	R	M	R	R	R	R
A. candidus	R	R	R	R	M	R
A. clavatus	R	R	R	M	R	-
A. flavus	V	M	M	M	V	R
A. fumigatus	-	-	R	R	R	R
A. longivesica	-	-	R	-	R	-
A. niger	M	V	M	R	M	R
A. sydowi	-	-	R	R	-	-
A. tamarii	R	R	R	-	R	-
A. terreus	R	R	R	M	R	-
A. versicolor	R	-	R	R	-	-
Eurotium repens	R	R	R	M	M	R
***Penicillium* species**						
P. chrysogenum	R	M	R	R	R	R
P. citreonigrum	-	-	R	-	R	-
P. expansum	-	-	R	-	R	R
P. islandicum	R	R	R	R	M	-
P. pinophilum	-	-	-	-	-	R
P. spinulosum	-	R	-	-	R	-
Eupenicillium hirayamae	-	R	-	R	R	-
Zygomycetes						
Absidia corymbifera	R	-	-	R	R	-
Cunninghamella polymorpha	R	-	R	R	R	R
Mucor circinelloide	R	R	R	R	M	-
Rhizopus arrhizus	M	M	M	M	R	-
Rhizopus microsporus	M	R	M	M	R	R
Syncephalastrum racemosum	M	R	R	R	R	-
Others						
Curvularia lunata	R	R	-	-	R	R
Dreschlera sp.	R	R	-	R	R	R
Moniliella sp.	R	-	R	R	-	R
Total no. of species	**20**	**18**	**22**	**21**	**24**	**14**
Moisture contents (% dry basis)	**9.30**	**10.14**	**6.76**	**5.75**	**6.67**	**6.39**

KEYS :

V : Very frequent occurring

M : Moderately frequent occurring

R : Rarely frequent occurring

based food as purchased from retail outlets before exposing to the different levels of A_w.

Twenty species were isolated on the initial composite samples of ordinary rice having an average initial moisture content of 9.30%. *Aspergillus flavus* occurred very frequently while that of *A. niger, Rhizopus arrhizus, R. microsporus* and *Syncephalastrum racemosum* were moderately frequent. The rarely occurring species were *A. aculeatus, A. candidus, A. clavatus, A. tamarii, A. terreus, A. versicolor, Eurotium repens, Penicillium chrysogenum, P. islandicum, Absidia corymbifera, Cunninghamella polymorpha, Mucor circinelloide, Curvularia lunata, Dreschlera* sp. and *Moniliella* sp..

Eighteen species were isolated on the initial composite samples of glutinous rice having an average initial moisture content of 10.14%. *Aspergillus niger* occurred very frequently while that of *A. aculeatus, A. flavus, Penicillium chrysogenum,* and *Rhizopus arrhizus* were moderately frequent. Other fungal species which occurred only rarely are *A. candidus, A. clavatus, A. tamarii, A. terreus, Eurotium repens, Penicillium islandicum, P. spinulosum, Eupenicillium hirayamae, Mucor circinelloide, R. microsporus, Syncephalastrum racemosum, Curvularia lunata* and *Dreschlera* sp.

Twenty-two species were isolated on riceflour having an average initial moisture content of 6.76%. At day 0, *Aspergillus flavus, A. niger, Rhizopus arrhizus* and *R. microsporus* were moderately frequent and other fungal species which occurred only rarely are *A. aculeatus, A. candidus, A. clavatus, A. fumigatus, A. longivesica, A. sydowi, A.tamarii, A. terreus, A. versicolor, Eurotium repens, Penicillium chrysogenum, P. citreonigrum, P. expansum, P.*

islandicum, P. spinulosum, Cunninghamella polymorpha, Mucor circinelloide, Syncephalastrum racemosum and *Moniliella* sp.

Twenty-one species were isolated on the initial composite samples of glutinous riceflour having an initial average moisture content of 5.75%. Species of *Aspergillus clavatus, A. flavus, A. terreus, Eurotium repens, R. arrhizus and R. microsporus* were moderately frequent occurring while that of *A. aculeatus, A. candidus, A. fumigatus, A. niger, A. sydowi, A. versicolor, Penicillium chrysogenum, P. islandicum, Eupenicillium hirayamae, Absidia corymbifera, Cunninghamella polymorpha, Mucor circinelloide, Syncephalastrum racemosum, Dreschlera* sp. and *Moniliella* sp. occurred only rarely.

Among the starch-based food studied, the highest number of fungal species (24 species) occurred on the initial composite samples of wheatflour having an average moisture content of 6.67 %. Incidence of *Aspergillus flavus* was very frequent while that of *Aspergillus candidus, A. niger, Eurotium repens, Penicillium islandicum and Mucor circinelloide* were moderately frequent. The rarely occurring species were *A. aculeatus, A. clavatus, A. fumigatus, A. longivesica, A. tamarii, A. terreus, P. chrysogenum, P. expansum, P. citreonigrum, P. spinulosum, Eupenicillium hirayamae, Absidia corymbifera, Cunninghamella polymorpha, Rhizopus arrhizus, R. microsporus, Syncephalastrum racemosum, Curvularia lunata* and *Dreschlera* sp.

On the other hand, the least number of fungal species occurred on the initial composite samples of cornflour having an initial average moisture content of 6.39%. Fourteen species occurred only rarely viz. *Aspergillus aculeatus, A. candidus, A. flavus, A. fumigatus, A. niger, Eurotium repens, Penicillium* *chrysogenum, P. expansum, P. pinophilum, Cunninghamella polymorpha, Rhizopus microsporus, Curvularia lunata, Dreschlera* sp. and *Moniliella* sp.

From the data presented in Table 4.2 - 4.7, fungal species occurring on each starch-based food can be categorized as dominant, important and minor species. Dominant species referred to species occurring at more than five sampling time for all levels of A_w, important species referred to species occurring at 3 - 5 sampling time for all levels of A_w and minor species referred to species occurring at less than 2 sampling time for all levels of A_w.

As indicated in Table 4.2, the dominant fungal species occurring on ordinary rice grains upon storage at various A_w levels are *A. candidus, A. flavus, A. niger, R. arrhizus* and *R. microsporus* and the important species being *A. corymbifera* and *P. chrysogenum* while the incidence of other species were only minor. *Aspergillus candidus* which occurred only rarely at day 0 becomes moderately or very frequent when ordinary rice grains is conditioned to A_w above 0.65. Other species not detected at day 0 but occurred at various A_w levels are *A. fumigatus, A. sydowi, P. aurantiogriseum, P. pinophilum, P. spinulosum, P. vinaceum, Eupenicillium hirayamae, Monascus mucoroides* and *Trichoderma* sp. Hence, an increase in water activity of ordinary rice grains from 0.25 to > 0.65 encourages proliferation of *A. candidus* and nine other fungal species. However, the total number of species detected at each A_w did not vary significantly from day 0.

As indicated in Table 4.3, the dominant fungal species occurring on glutinous rice grains upon storage at various A_w levels are *A. flavus, A. niger, R. arrhizus* and *R. microsporus* while *A. candidus, P. chrysogenum* and *P.*

cont. Table 4.2 The incidence of fungi on ordinary rice stored at different levels of A_w at 25 °C for 96 days

Water Activity (A_w)		0.65	0.75	0.80	0.85	0.90	0.95	0.98
Days of storage	0	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96
FUNGAL SPECIES								
***Penicillium* species**								
P. aurantiogriseum Dierckx.	-	- R - - - - - - -	- - - - - - - - -	- R - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. chrysogenum Thom. *	R	- R - - R - - M R	- R R - R R - R M	- - - - - - R R R	- R - R R R R - -	- - - R R R R R -	R R - - R R R - -	- R - R R R R - -
P. islandicum Sopp.	R	R - - - - - - - R	- R R R - - - - R	- R R - - - - R R	- R - R - R - R V	- M R R - - - - M	- R - R - - - R V	- R R - - R - - M
P. pinophilum Hedgcock.	-	- - - - - - R - -	- R - R R R R - R	- - - - R - - - -	- - - R - - - - -	- - - - R - - - -	R - R - R R R - -	- - - - - - - - R
P. spinulosum Thom.	-	- - - - - - - - R	R - - - - - - - -	- - - - - - - - R	- - - - - - - - -	- - - - - - - - R	- - - - - - - - -	- - - - - - - - R
P. vinaceum J. Gilman & E.J. Abbot.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - R R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
Eupenicillium hirayamae D. B. Scott & Stolk.	-	- - - - - - - - -	- R R - - - - R -	- - - R - - - - R	- R - - - - - - -	- - M R R - R R -	- - R - - - R - -	- - - - - - - - R
Others								
Curvularia lunata (Wakker)	R	- - - - - - - - -	- - - - - - - - -	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
Dreschlera sp.	R	- R - - - - - - -	- - - - - - - - -	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- R - - - - - - -
Monascus mucoroides Van Tiegh..	-	- - - - - - - - -	- R - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
Moniliella sp.	R	R - - - - - - - -	- - - - R - - - -	- - R - - - - - -	M - R R - - - - -	- - - - - - - - R	- - - R R - - - -	- - - R R - - - -
Trichoderma sp.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	R - - - - - - - -
Total no. of species detected	**20**	**23**	**22**	**23**	**19**	**20**	**21**	**23**

Keys :

V : very frequent occurring
M : moderately frequent occurring
R : rarely frequent occurring

** : Dominant species ; occurring at > 5 sampling time at all A_w levels.
* : Important species ; occurring 3 - 5 sampling time at all A_w levels.
no asterisk : Minor species ; occurring ≤ 2 sampling time at all A_w levels.

Table 4.3 The incidence of fungi on glutinous rice stored at different levels of A_W at 25 °C for 96 days

Water Activity (A_w)		0.65	0.75	0.80	0.85	0.90	0.95	0.98
Days of storage	0	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96
FUNGAL SPECIES								
***Aspergillus* species**								
A. aculeatus Iizuka.	M	- R - R - - - - -	R - - - R - R - -	R - - - - R - R -	R - - R R - R R -	- R - R - - R - -	R - - - - - - - -	R R - - - - - - -
A. candidus Link. *	R	- R - - - - R M M	R - - - - R R R M	- - - - - R M M M	- R - - - - M V V	R - - - - R M M V	- R R - - - M M M	- R - - - R V M M
A. clavatus Desmazieres.	R	- - - - - - R R -	- - - - R - - - -	- - - - - - R - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - R - -
A. flavus Link. **	M	R M M M M M M V M	M V M V M V V M M	R M M M M V M M R	R R R M M V M V M	R R R M M V R M R	M M M M M V V M R	R M R M M V R V M
A. fumigatus Fresenius.	-	- - - - - - - - -	- - R - - - - - -	- - - - R - - - -	- - - - - - - - -	- R - - - - - - R	- - - - - - - - -	- - - - - - - - -
A. longivesica L. H. Huang & Raper.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- R - - - - - - -	- - - - - - - - -
A. niger Van Tieghem. **	V	M M M M M M M M M	V M M M V M M M M	M M M M M M M M M	M M V V M M M M M	R M V M M M M M M	M R M M M M M R R	R M V M M M M M M
A. sydowi (Bain. & Sart.) Thom & Church.	-	- - - - - - - - R	- - - - - - - - R	- R - - - - - - M	- - R - - - - R M	- - - R - - - - R	- R - - - - - - R	- - - - - - - R R
A. tamarii Kita.	R	- - - - - - - - -	R R R - R - - - -	- - - - - - - - R	- - - - - - R R -	- - - - - R - - -	- - - - - - - R R	- - - - - - - R -
A. terreus Thom.	R	- - - - - - - - -	- - - - - R - - -	- - - - - - - - -	- - - - - - - - R	- - R - - - - - -	- R - - - - - - -	- - - - - - - - -
A. versicolor (Vuill.) Tiraboschi.	-	R - - - - - - - -	- - - - - - - - -	- - - - - - - - R	- - - - - - - R -	- - - - - - - R -	- - - - - - - - -	R - - R - - - R -
Eurotium repens de Bary.	R	R - - - R R R M R	- - - - - - R R R	- R - - - - M R R	R - - - R R - V R	R - - - - R R M R	R R - - R R R R	R - - - - M - - -
Zygomycetes								
Absidia corymbifera (Cohn) sacc. & A. Trotter.	-	- - - R - - - - R	- - - R R - - - R	- - - - - - - - -	R - R M R R M R M	- - - M R R - R R	M R - R - R R R R	R - - R R R R R -
Cunninghamalla polymorpha Pispek.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	R - - - - - - - -
Mucor circinelloides Tiegh.	R	- - - R - R - - -	- - - - - - - - -	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	R - - - - - - - -
Rhizopus arrhizus A. Fisch. var. *arrhizus* Ellis. **	M	R R M R M M M M R	R R M R M M M R M	R M R M M M M R R	R R - M R R R R -	- R - M R M M R R	R R R M M R R M R	R R R M M M - R R
R. microsporus Tiegh. **	R	R R M M R M R R R	R R R M M M M M M	R M M M M M M R M	- R R R R R R R R	R M R R R M M R -	R R M R M R R R -	M R M R R M R R R
Syncephlastrum racemosum Cohn ex Schroter.	R	- - R - R R R R -	- - R R - R R R R	- - M R - - R R R	M - R - - - M R -	- - - - R R - - -	- - R R R R R - -	R R R - M R R R R

cont. Table 4.3 The incidence of fungi on glutinous rice stored at different levels of A_w at 25 °C for 96 days

Water Activity (A_w)		0.65	0.75	0.80	0.85	0.90	0.95	0.98
Days of storage	0	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96
FUNGAL SPECIES								
***Penicillium* species**								
P. aurantiogriseum Dierckx.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - R - - - -
P. chermesinum Biourge.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. chrysogenum Thom. *	M	- R - R - R R R R	- - - - R R - M M	- M R - M R R M R	- R R R R - R V R	- M R - - R M M V	R R R - R M V M -	- M M R - R V V R
P. citrinum Thom.	-	- - - - - - - - -	- - - - - - - R -	- - - - - - R - -	- - - - - - - - -	- - - - - - - - -	- - - - - - R - -	- - - - - - - - -
P. expansum Link.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - R -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. islandicum Sopp. *	R	R R R R - - - - R	R R R M - R - - M	- M R R R R - - R	R R M R R R M M M	- M R R - R M R V	R R R R - R M R V	- R R R R R R M R
P. minioluteum Dierckx.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - R - - R - -
P. oxalicum Currie & Thom.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - R -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. pinophilum Hedgcock.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- R - R - - - - -	- - R R - - - - -	- - - - - - - R -	- - R - - R - - -
P. rugulosum Thom.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - R - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. spinulosum Thom.	R	- - - - - - - R -	- R - R - - - - R	- - - R - - - R -	- - - - - R - - -	- - - R - - - - R	- - - R - - - R -	- - R - R - - R R
P. thomii Marie.	-	- - - - - - - - R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
Eupenicillium hirayamae D. B. Scott & Stolk.	R	- - - - - - - - R	- - - - - - - - -	- - R - - - - - -	- - - - - - - - -	- - - - - - - - -	- R - - - - - - -	- - - - - - - - R
Eupenicillium sp.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- R - - - - - - -	- - - - - - R - -
Others								
Curvularia lunata (Wakker) Boedijn.	R	- - - - - - - - -	R - - - - - - - -	R - - - - - - - -	- R - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
Dreschlera sp.	R	- R - - - - - - -	- - - - R - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	R - - - - - - - -
Moniliella sp.	-	- - - - - - - - -	- - - - - - - - -	- - - R - - - R -	- - - - - - - - -	- - - - - - - - -	- R - - - - - - -	- - - - - - - - -
Trichoderma sp.	-	- - - - - R R - -	- - - - - - - - -	- - - - - - - - R	- R - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - R - - - - - -
Total no. of species detected	**18**	**20**	**20**	**24**	**20**	**18**	**21**	**25**

Keys :

V : Very frequent occurring

M : Moderately frequent occurring

R : Rarely frequent occurring

** : Dominant species ; occurring at > 5 sampling times at all A_w levels.

* : Important species ; occurring at 3 - 5 sampling times at all A_w levels.

no asterisk : Minor species; occurring at ≤ 2 sampling times at all A_w levels.

islandicum are important species. Conditioning of glutinous riceflour from a water activity of 0.36 to water activities > 0.65 also encourages proliferation of other species not detected at day 0 i.e. *A. fumigatus, A. longivesica, A. sydowi, A. versicolor, A. corymbifera, C. polymorpha, P. aurantiogriseum, P. chermesinum, P. citrinum, P. expansum, P. minioluteum, P. oxalicum, P. pinophilum, P. rugulosum, P. thomii, Eupenicillium* sp., *Moniliella* sp. and *Trichoderma* sp. The total number of species detected at 0.80 and 0.98 A_w was slightly more than the initial sample at day 0.

As indicated in Table 4.4 the dominant fungal species occurring on riceflour upon storage at various A_w levels are *A. flavus, A. niger, A. terreus, R. arrhizus, R. microsporus* and *P. chrysogenum* while important species are *A. candidus, A. clavatus* and *Moniliella* sp. *Aspergillus terreus* and *P. chrysogenum* which occurred only rarely initially becomes moderately to very frequently occurring at higher water activity levels. Also, conditioning riceflour from 0.29 A_w to a water activity > 0.65 encourages proliferation of fungal species not detected initially i.e *A. corymbifera, P. aurantiogriseum, P. citrinum, P. griseoroseum, P. minioluteum, P. pinophilum, P. spinulosum, P. variabile, P. vinaceum, Eupenicillium hirayamae, Eupenicillium* sp., *Dreschlera* sp., *Monascus mucoroides* and *Trichoderma* sp. The total number of species detected at higher water activity levels was more than at day 0.

As indicated in Table 4.5 the dominant fungal species occurring on glutinous riceflour when stored at higher A_w levels are *A. flavus, R. arrhizus, R. microsporus* and *P. chrysogenum* while the important species are *A. candidus, A. fumigatus, A. terreus* and *Eurotium repens*. *Aspergillus niger* which occured

Table 4.4 The incidence of fungi on riceflour stored at different levels of A_W at 25 °C for 96 days

Water Activity (A_w)		0.65	0.75	0.80	0.85	0.90	0.95	0.98
Days of storage	0	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96
FUNGAL SPECIES								
***Aspergillus* species**								
A. aculeatus Iizuka.	R	- - - - R - R - -	- - - - - - - - -	R M - R - R - - -	- R - - R - - - R	M R - - - - - R -	R R - R R - - - -	- R - - - - - - -
A. candidus Link. *	R	R M - R R - M R M	R R - - - - M M R	R M R R R R V M M	- R - R R R M M M	R R R M R M M M -	R R M R R - V R -	M R M - R R R R -
A. clavatus Desmazieres. *	R	M R R M R R R R R	- - - R - - - R R	M M R R R R - R M	R - R R - - - R R	R - R M - R R - -	R R R R R - - - -	R R R - - - - - -
A. flavus Link. **	M	R R R R M V V M V	R M M M M V V M M	R M M M M M M M V	M M M M M V M V M	R M M M M V V M V	- R R M M R M - -	R R M M V M R - -
A. fumigatus Fresenius.	R	- - - - R R - - -	M R M R V M R R R	R - - R R - R - -	M R R R - R R - -	- - - R R - R - R	- - - - R - - - -	- - - R R - - - -
A. longivesica L. H. Huang & Raper.	R	M - - - - - - - R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	R - - - R - - - -	- R - - - - - - -	- - - - - - - - -
A. niger Van Tieghem. **	M	M M R M M M R M M	M M M M R R M M R	M M M M M M V M M	M M M M M R M R V	R M M M M M M M M	M M M R M M R - -	R M M M R M - - -
A. sydowi (Bain. & Sart.) Thom & Church.	R	- - - - - - - - -	- R - - - - - R R	- R - - - - - R R	- - - - - - - - -	- R - - - - - - -	- R - - R - - - -	- R - - - - - - -
A. tamarii Kita.	R	- - R - - - - - -	- R - - - - - - -	- - - - R - R - R	- - - - - - R - -	- - - - - - - - -	- - - - - - - - -	- - - - R - - - -
A. terreus Thom. **	R	M M M M V M M M M	V R R M M R M - R	M M R R V V R R M	R R - M R M M M V	R R M V V M R - M	M M V R V R R - -	M M V R R R R - -
A. versicolor (Vuill.) Tiraboschi.	R	R - - R R - - - -	- - - - - - - - -	- - - - - - - - -	- - - R - - - - -	- - - - R - - - -	R - R R - - - - -	- - - - - - - - -
Eurotium repens de Bary.	R	R R - - - - R R R	- - - - - R R M R	- - - - - - - R -	- R R - R R - R -	R - - - R R - - -	- - - - R - - - -	- - R R - - R - -
Zygomycetes								
Absidia corymbifera (Cohn) sacc. & A. Trotter.	-	M - - R R - R - R	- R M R R R M - R	- - - - R - R - -	- R R R - - R R -	- - - - - - - - -	- - - R - - - - -	- - - - - - R - -
Cunninghamalla polymorpha Pispek.	R	- - - - - - - - -	- - - R - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - R - - - - -	- - - - - - - - -
Mucor circinelloides Tiegh.	R	- - - - - - - - -	- - R - - - R - R	- - - - - - R - -	- - R - - - - - -	- - - - - - - - -	V M R - R - - - -	- R R - - R - - -
Rhizopus arrhizus A. Fisch. var. *arrhizus* Ellis. **	M	R R M M M M M M M	R M M R M M M R M	M M M M V M M M M	R R M M M M V M M	M R M M M M M V M	M R M R M R - -	R R M R R R - - R
R. microsporus Tiegh. **	M	M R R M R M R M R	R R M V M M R M M	M R - M R R R R R	R M M M M M R R R	R R R R R M - R R	R R R R M R R - -	R R R R R M - - -
Syncephlastrum racemosum Cohn ex Schroter.	R	- - R - - - R R -	R - - R - - - R -	R - - - R R - - -	R - - R - - R - -	R - - - - R R - R	- M R - - R R - -	- - - - - R M - -

cont. Table 4.4 The incidence of fungi on riceflour stored at different levels of A_w at 25 °C for 96 days

Water Activity (A_w)		0.65	0.75	0.80	0.85	0.90	0.95	0.98
Days of storage	0	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96
FUNGAL SPECIES								
***Penicillium* species**								
P. aurantiogriseum Dierckx.	-	- - - - - - - - -	- - - - - - - R -	- - - - - - - - -	- - - - - - - - -	- - - - - R - - -	- - - - - - - - -	- - - - - - - - -
P. chrysogenum Thom. **	R	M R R M R M V R R	- R - R R - M M M	M M R M R R V M R	R M - M R R M M M	R M R R R M V M R	M M M M M R M M -	R R V M V M R M -
P. citreonigrum Dierckx.	R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - R - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. citrinum Thom.	-	- - - - - - - R -	- - - - - - - - -	- - - - - - - - -	- - - - R - - R -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. expansum Link.	R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. griseoroseum Dierckx.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - R - - - - -	- - - - - - - - -
P. islandicum Sopp.	R	- M M M M M R R R	- - - R - R - - -	M R R R R R R - -	- R R R M M R M R	R R M M R R R R -	- M M R R R R M M	M R R R R M - V V
P. minioluteum Dierckx.	-	- R - - - - - - -	- - - - - - - R -	- - - - - - - - -	- - - - - - - - -	- - - R - - - - -	- R - R R - - - -	- - - - - - - - -
P. pinophilum Hedgcock.	-	- - - R R - - - -	- - - R R - - R -	- - R - R R R - -	- - - - R - - - -	- - - - - - - - R	- - - - R - - - -	R - - - - R - - -
P. spinulosum Thom.	-	R R R R - R - - -	- - - - - - R - -	- R - - R R - - R	- - - - R - - - R	R R - R - - R - -	- R - - - R R - -	R R R - - - - - -
P. variabile Sopp	-	- - - - - - - - -	- - - - - - R R -	- - - - - - R R -	- - - - - - R R -	- - - - - - - R -	- - - - - - R - -	- - - - - - - - -
P. vinaceum J. Gilman & E.J. Abbot.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - R - - - -	- - - - - - - - -
Eupenicillium hirayamae D. B. Scott & Stolk.	-	- - - - - - - - R	- - - - - - - - -	- - - - - R R - R	- - R - - R R - -	- R R - - - R M V	- - R - - - R R R	- - - - - R R - R
Eupenicillium sp.	-	- - - - - - - - R	- - - - - - - - -	- - - - - R R - R	- - - - - - R - -	- R R - - - R - M	- - R - - - R R R	- - - - - - R - R
Others								
Dreschlera sp.	-	- - - - - - - - -	- R - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	M - - - - - - - -	- - - - - - - - -
Monascus mucoroides Van Tiegh.	-	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - R - - -	- - - - - - - - -	R - - - - - - - -
Moniliella sp. *	R	M R M M M R R M M	- R R R R R - - M	R R M R M R M R M	- - - R R - R R M	R R R R R R R R M	M R M R R V R - -	M R M R M M - - -
Trichoderma sp.	-	- - - - - - - - -	- - - - - - - - -	R R - - - - - - -	- R - - - - - - -	R - - - - - - R -	- R - - - - - - -	- R R - R - - - -
Total no. of species detected	**22**	**29**	**24**	**24**	**26**	**26**	**30**	**24**

Keys :
V : Very frequent occurring
M : Moderately frequent occurring
R : Rarely frequent occurring

** : Dominant species ; occurring at > 5 sampling times at all A_w levels.
* : Important species ; occurring at 3 - 5 sampling times at all A_w levels.
no asterisk : Minor species; occurring at ≤ 2 sampling times at all A_w levels.

Table 4.5 The incidence of fungi on glutinous riceflour stored at different levels of A_W at 25 °C for 96 days

Water Activity (A_w)		0.65	0.75	0.80	0.85	0.90	0.95	0.98
Days of storage	0	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96
FUNGAL SPECIES								
***Aspergillus* species**								
A. aculeatus Iizuka.	R	R R - - - - R - -	- - R - - - - - -	- R - - - - - - -	- R - - R - R M -	R - - - - - - R -	R - R R R - - - -	- R R - - - - - -
A. candidus Link. *	R	R - - R - - R R M	R R - R R R V M M	R R R R - - M M M	- - - - - - M M M	R - - - R - M M M	R R R - - R M - R	- R R - - R R R -
A. clavatus Desmazieres.	M	R - - - - R - R -	- R - - - - - - -	R - - - R - - - -	R R - - R - - - R	R - R - - - - - -	R - - - R - - - -	- - - - - - - - -
A. flavus Link. **	M	R M M V R M M M M	R M M M M V M V M	R R R R M V M M M	M M M M M M V V M	R M M V M M V M M	R R M M V V M - -	R R M M M V M R R
A. fumigatus Fresenius. *	R	M R R - - M - R R	- - R R R R R R R	R R - - R - R R R	- M M R V R R - R	M M M M R R R - -	- - R R R - - -	R R R R R R - - -
A. longivesica L. H. Huang & Raper.	-	- - R - - - - - -	- - - - - - - - -	R - - - R - - - -	- R - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
A. niger Van Tieghem. **	R	M M M M R M M M M	R M M M M M M M M	R M M M M M M R M	R R M M M M M M M	R M M R M M M M R	M M M M R R M - -	R M M M M M - - R
A. sydowi (Bain. & Sart.) Thom & Church.	R	- - R - - - - - -	- - R - - - - R -	- M - R - - - R R	- R R - - - - R R	- - - - - - - R R	- - - - - - - - -	- R - - - - - - -
A. tamarii Kita.	-	- - - - R - - - R	- - - R R R - - -	- - - R - R - - -	- - - - - R R - -	- - R R - - - - -	- - - - - - - - -	- - - - - - - - R
A. terreus Thom. *	M	V M R M - M R M M	R R M M M R R - R	R - R M R R - R R	R R R M M M R R R	R M R V M M M R R	R M - R R - R - -	V R M R R R - R -
A. versicolor (Vuill.) Tiraboschi.	R	R - - - - - - - -	- - - - - - R - -	- - - - - - R - -	- - - - - - R - -	- - - - - - - - -	- R - - - - - - -	- - - - - - - - -
Eurotium repens de Bary. *	M	R R R - R - R M R	M R R R R R R M M	R R R R R R R R R	R R R R R R - - -	M R R R R R R - -	R R R R R R - - -	R M R R - - - - -
Zygomycetes								
Absidia corymbifera (Cohn) sacc. & A. Trotter.	R	- - - R - - - R R	- - R R - - - - -	- - - - M R - - -	- - - R - - - - -	- - - R - - - - -	- - - - R - - - -	- R - - - R - - -
Cunninghamalla polymorpha Pispek.	R	- - - R - - - - -	R - R R - - R - -	- - R R - - - - -	- - - R - - - - R	R - R - - - - - -	- - R - - R - - -	- - - - - - - - -
Mucor circinelloides Tiegh.	R	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - R - - - -	- - - - - - - - -	- - - - - - - - -	- - - R - - - - -
Rhizopus arrhizus A. Fisch. var. *arrhizus* Ellis. **	M	R R M M - M M M M	R R M M M R R M M	M R V M M M M M M	V M M M M M M V M	R M M M R M M M R	M M M V M M R R R	R R M M M R - - -
R. microsporus Tiegh. **	M	R M M M R M M M R	R R M R M M M M M	M R R M R M M M R	M M M M M M M M M	R M M M M M R M M	M R M M R R R - -	R R M M M R - - -
Syncephlastrum racemosum Cohn ex Schroter.	R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -

cont. **Table 4.5 The incidence of fungi on glutinous riceflour stored at different levels of A_w at 25 °C for 96 days**

Water Activity (A_w)		0.65	0.75	0.80	0.85	0.90	0.95	0.98
Days of storage	0	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96
FUNGAL SPECIES								
***Penicillium* species**								
P. aurantiogriseum Dierckx.	-	- - - - - - - - - - -	- - - - - - - - R -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- R - - - - - - - - -	- - - - - - - - - - -
P. chrysogenum Thom. **	R	R R R M M R – – R	– R – R – V V M R	R M M – R – R M M	R R – R R – M M M	R R M R M M V M M	R R M R M R V M –	R R R M M M M V –
P. citreonigrum Dierckx.	-	- - - - - - - - - - -	– R - - - - - - R -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - R	- - - - - - - - - - R
P. citrinum Thom.	-	- - - - - - R – – R	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - R – –	- - - - - R – – R	- - - - - - - - - - R
P. islandicum Sopp.	R	R R R R – R R – –	R M R R – R R – –	– R R R R R – – R	– R - - - - - - R –	– R R R R R – R R	– R R – – – R M V	– M R R R – M M M
P. minioluteum Dierckx.	-	- - - - - - - - - - -	- - - - - - - - R -	- - - - - - - - - - -	- - - - - - - - - - -	– R - - - - - - - - -	- - - R - - - - - - -	- - - - - - - - - - -
P. pinophilum Hedgcock.	-	– R R – – – – R –	– – – R R – – – –	R R R R – R – – –	– R - - - - - - - - -	R R R – – R – – –	– – R R – – – – –	– R – – – R – R –
P. purpurogenum O. Stoll.	-	- - - - - - - - - - -	- - - - - - - - - - -	– R - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -
P. spinulosum Thom	-	– R - - - - - - - - -	– R R - - - - - - -	– – R – – M R – –	– – – R - - - - - -	– – R – R – – – –	– – M – – – R M –	– – R – – – R M –
P. thomii Marie.	-	– – R - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -
P. variabile Sopp.	-	– – – – R – R R –	– – – R – – R – –	- - - - - - - R – –	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -
P. vinaceum J. Gilman & E.J. Abbot.	-	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - R –	– – – – R – – – –	– – – – R R – – –	- - - - - - - - - - -
Eupenicillium hirayamae D. B. Scott & Stolk.	R	– – R R – – – R –	– R R – – – R R –	– R R R R R R – –	– – R R – R – R R	– – – – – – R R – –	– R – R – – R M R	– R R – – – V M R
Eupenicillium sp.	-	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - R	- - - - - - - - - - R	- - - - - - - - M –	- - - - - - - - R –
Others								
Curvularia lunata (Wakker) Boedijn.	-	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	R - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -
Dreschlera sp.	R	- - - - - - - - - - -	R - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	– R - - - - - - - - -	- - - - - - - - - - -
Moniliella sp.	R	M R - - - - - - - - -	– – – R – – R R R	M – R R – – R – M	– R – R - - - - - -	R – – R – – V V –	R - - - - - - - - - -	– R R R – R – – –
Trichoderma sp.	-	- - - - - - - - - - -	- - - - - - - - - - -	- - - - - - - - - - -	- - - - R - - - - -	– R - - - - - - - - -	– R - - - - - - - - -	- - - - - - - - - - -
Total no. of species detected	**21**	**26**	**26**	**24**	**26**	**26**	**27**	**22**

Keys :

V : Very frequent occurring

M : Moderately frequent occurring

R : Rarely frequent occurring

** : Dominant species ; occurring at > 5 sampling times at all A_w levels.

* : Important species ; occurring at 3 - 5 sampling times at all A_w levels.

no asterisk : Minor species ; occurring at $\leq$ 2 sampling times at all A_w levels.

rarely initially on glutinous riceflour having a water activity of 0.30 becomes dominant when conditioned to a water activity > 0.65 and proliferation of *A. longivesica, A. tamarii, P. aurantiogriseum, P. citreonigrum, P. citrinum, P. minioluteum, P. pinophilum, P. purpurogenum, P. spinulosum, P. thomii, P. variabile, P. vinaceum, Eupenicillium* sp., *Curvularia lunata* and *Trichoderma* sp. also occurred. Hence, the total number of species detected at higher A_w was more than at day 0.

As indicated in Table 4.6, the dominant fungal species occurring on wheatflour are *A. candidus, A. flavus* and *P. chrysogenum* while important species are *A. niger, Eurotium repens, R. arrhizus* and *Absidia corymbifera.* Fungal species which were detected after wheatflour was conditioned from a water activity of 0.31 to > 0.65 are *A. versicolor, P. aurantiogriseum, P. citrinum, P. griseofulvum, P. minioluteum, P. pinophilum, P. simplicissimum, P. variabile, P. verrucosum, Eupenicillium* sp. and *Trichoderma* sp. Compared to day 0, the total number of species detected was more at A_w 0.65, 0.75, 0.85 and 0.90 and less at other A_w values.

As indicated in Table 4.7, the only dominant fungal species occurring on cornflour is *P. chrysogenum* while *A. flavus* and *A. niger* represents the important microflora. *P. chrysogenum* which occurred only rarely initially becomes moderately or very frequent when the water activity of cornflour was increased from 0.30 to > 0.65. After storage at high water activity levels proliferation of species not detected initially also occurred i.e. *A. clavatus, A. longivesica, A. sydowi, A. tamarii, A. terreus, A. versicolor, Absidia corymbifera, Mucor circinelloide, R. arrhizus, Syncephalastrum racemosum, P. aurantiogriseum, P.*

cont. Table 4.6 The incidence of fungi on wheatflour stored at different levels of A_w at 25 °C for 96 days

Water Activity (A_w)		0.65	0.75	0.80	0.85	0.90	0.95	0.98
Days of storage	0	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96
FUNGAL SPECIES								
Penicillium species								
P. aurantiogriseum Dierckx.	-	- R - - - - R - -	- - - - - - - R -	- - - - - - - - -	- R - - - - - R -	- R R - - - - - -	- R - - - - - - -	- - - - - - - - -
P. chrysogenum Thom. **	R	- M V M V M M M M	M M R R - - M R	R M R R R R M R M	- M M V M R M M M	- M R R M M M R R	R M M R R M - - R	- M R R M M - R R
P. citreonigrum Dierckx.	R	- R R - - R R - -	- - - - - - - - R	- R - - - - - - -	- - - - R - - - -	R R - R - - - R -	- R - - - - - - -	- - - - - - - R -
P. citrinum Thom.	-	- - - - - - - R -	- - R - - - - - -	- R - - - - - - -	- - - - - R - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. expansum Link.	R	- R - - - - - R -	- - - - - - - - -	- - - - - - - - -	- - - - R - - - -	- R R - R R R - -	- - - - - - - - -	- - - - - - - - -
P. griseofulvum Dierckx.	-	- - - R R - - - R	- R - - - - - - -	- - - - - - - - -	- R - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. islandicum Sopp.	M	- R - - R - - R -	- R - - - - - - -	- R R - - - - - -	- R R R - - R R -	- - R - R R - R -	- - R - - - - - -	- R - R R R - - -
P. minioluteum Dierckx.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - R - - - - - -	- - - - - - - - -	- - - - - - - - -
P. pinophilum Hedgcock.	-	- R R - R - R - R	- R - - - - - - -	- - R R - - - - -	- R R R - - - - R	- R R R R R - - R	- - - - - - - - -	- - R - - - - - -
P. simplicissimum (Oudem.) Thom.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - R - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. spinulosum Thom.	R	- R M R R M M R R	- R R - - - - - -	- - - - - - - - -	- - M R R M M R R	- M M R R - R - -	- R M - R - - - -	- R - - - - - - R
P. variabile Sopp.	-	- - - - - - - - -	- - - - - - R - -	- - - - - - - - -	- - - - - - - - R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. verrucosum Dierckx.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- R - - - - - - -	- - - - R - - - -	- - - - - - - - -	- - - - - - - - -
Eupenicillium hirayamae D. B. Scott & Stolk.	R	- R R M V M V R V	- R - - - - R - - R	- - - - - R - - -	- - R M V M M R V	- - R V M M M M M	- R R - R - R - -	- M R - - R R R R
Eupenicillium sp.	-	- - R R M M M - R	- R - - - - - - -	- - - - - R - - -	- R - M V M R - R	- - R M M M R R R	- R R - - - - - -	- M - - - R - R -
Others								
Curvularia lunata (Wakker) Boedijn.	R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	R - - - - R - - -	R - - - - - - - -	- - - - - - - - -
Dreschlera sp.	R	- R - - - - - - -	R - - - - - - - -	- R - - - - - - -	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
Trichoderma sp.	-	- - - - - - - - -	- - - - - - - - -	R - - - - - - - -	- - - - - - - - -	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -
Total no. of species detected	**24**	**28**	**25**	**22**	**30**	**26**	**22**	**21**

Keys : V : Very frequent occurring
M : Moderately frequent occurring
R : Rarely frequent occurring

** : Dominant species ; occurring at > 5 sampling times at all A_w levels.
* : Important species ; occurring at 3 - 5 sampling times at all A_w levels.
no asterisk : Minor species; occurring at ≤ 2 sampling times at all A_w levels.

Table 4.7 The incidence of fungi on cornflour stored at different levels of A_W at 25 °C for 96 days

Water Activity (A_w)		0.65	0.75	0.80	0.85	0.90	0.95	0.98
Days of storage	0	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96
FUNGAL SPECIES								
***Aspergillus* species**								
A. aculeatus Iizuka.	R	R R R - - M M R -	R M - R R R M R -	R R R - R R R - -	R R M - R R V R R	V R R R - R R - -	M - R R - R - - -	- R M R - - - R -
A. candidus Link.	R	- - - - - - R M V	- - - - - - V M V	- - - - - - V V V	- - - - - - V V M	- - R - - - M M V	- - R - - - R - R	- R - - - - - - -
A. clavatus Desmazieres.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	R - - - - - - - -
A. flavus Link. *	R	R R R R R M M M M	- - M R M M V M M	- - - R M M M M M	M - - M M M M M R	- - - R R M V V R	- R - R R R M R R	- - - R M M R - R
A. fumigatus Fresenius.	R	- R R - - - - - R	R R - - - - - - -	- M M R R - - - R	- R R - - M M - R	R R R - - - - - -	R - R R - - - - -	R - - R - - - - -
A. longivesica L. H. Huang & Raper.	-	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
A. niger Van Tieghem. *	R	- R - R - R R R R	- - - - R R R R R	- R - - - R M R -	M R - R R - R M R	M R - - M M V M -	R - - R R R R - -	- R - - R M R - R
A. sydowi (Bain. & Sart.) Thom & Church.	-	- - - - - - - M R	- R - - - - - M R	- R - - - - - V R	- R - - - - - R -	- - R R - - - R -	- R R - - R - - -	- - - - - - - - -
A. tamarii Kita.	-	- - - - - - - - -	- - - - - R - - -	- - - - - - R - -	- - - - R - - - -	R - - - - - - - -	- - - - - R - - -	- - - - - - - - -
A. terreus Thom.	-	- - - - - - - - -	- R - - - - - - -	- - - R - - - - -	- - - - - - - R -	- - - - - - - - R	- - - - R - - - -	- - - - - - - - -
Aspergillus versicolor (Vuill.) Tiraboschi.	-	- - - - - - - - -	- - - - - - - R -	- - - - - - - R -	- - - - - - - - -	- R R - - - - - -	- - - - - - - - -	- - - - - - - - -
Eurotium repens de Bary.	R	R R - - - M M M V	- R R - M R R M M	- R - R - M - - R	R - R - M R - R -	R R - - M M - - -	- - - R M - - - -	R - - - - - - - -
Zygomycetes								
Absidia corymbifera (Cohn) sacc. & A. Trotter.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - R - - - - -	- - - - - - - - -	- - - - - - - - -
Cunninghamalla polymorpha Pispek.	R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- M - - - - - - -	- - - - - - - - -	- - - - - - - - -
Mucor circinelloides Tiegh.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	R - - - - - - - -	R - - - - - - - -
Rhizopus arrhizus A. Fisch. var. *arrhizus* Ellis.	-	R - - R - R - - R	- - - - - - - R -	R R - R - - - - R	R R - R - - R R R	- R - M - - M M R	R - R R - R R - -	- R R R - - - - -
R. microsporus Tiegh.	R	- R - - - - - - -	R - R - - - - - -	- - - - - - - - -	- R - - - - - - -	- - - - - - - - -	R R - - - - - - -	R - - - - - - - -
Syncephlastrum racemosum Cohn ex Schroter.	-	- - - R - R - - -	- - - - - - - - -	- - R - - - - - -	- - - - - - - - -	- - - - R - R - -	- R - - R - - - -	- - - R - - - - -

cont. **Table 4.7 The incidence of fungi on cornflour stored at different levels of A_w at 25 °C for 96 days**

Water Activity (A_w)		0.65	0.75	0.80	0.85	0.90	0.95	0.98
Days of storage	0	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96	3 7 12 19 26 33 54 75 96
FUNGAL SPECIES								
***Penicillium* species**								
P. aurantiogriseum Dierckx.	-	R - - - - - R R -	- - - - - R R - -	- - - - - - M - -	R - - - - - R R -	- - - - - - R - -	- - - - R R R R -	- - - - - R - - -
P. chrysogenum Thom. **	R	R R R M R M M V V	R R R M R M M V V	- R - R M M M V V	M M M V V V M V M	R R M V V M M V V	- V M M M V M V M	R M V R V V R M M
P. citreonigrum Dierckx.	-	- - - - R - R - -	R - - - - - - - -	- - - - - - - - -	- - - - - R - - -	R - - - - - - - -	- - - - - - - - -	- - - R R - - - -
P. citrinum Thom.	-	- - - R - - - - -	- - - - - - - - R	- - - - - - - - R	- - - - R - - - -	- - - - - R - - R	- - - R R R - - R	- - - - - - - - -
P. expansum Link.	R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. griseoroseum Dierckx.	-	- - - - - - R R -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. islandicum Sopp.	-	- - - - - - - R R	- R - - - - - - R	- R - - - R - - R	- - - R - - - R -	- - - - - - - R R	- - - - - - R R -	- - - R R - - - -
P. minioluteum Dierckx.	-	- - - - - - - - -	- R - - - - - - -	- - - - - - - - -	- R - - - - - - -	- R - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. pinophilum Hedgcock.	R	- - - - - - - - -	- R - - - - - - -	- R - - - - - - -	R R R - - - - - -	- R R - R - - R -	- - - - - - - - R	- R - - - - - - -
P. purpurogenum O. Stoll.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - R - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - R
P. simplicissimum (Oudem.) Thom.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - R - - - - -	- - - - - - - - -	- - - - - - - - -	- - R M - - R - -
P. spinulosum Thom.	-	- - - - - - - R -	- - - - - - - - -	- - - R - - - R -	- - - R - - - - -	- - - - - R R - -	- - - R - R - - -	- - - - - R - - -
P. thomii Marie.	-	- - - - - - - - -	- - - - - - - R -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
P. variabile Sopp.	-	- - - R - - R M -	- - R - - R R R -	- - - - - - - R -	R - - R - R M R -	- - - R R - R R -	- - - M - R M - -	- - - M - - M R R
P. verrucosum Dierckx.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - R - - - -	- - - - - - - - -	- - - - - - - - -
P. vinaceum J. Gilman & E.J. Abbot	-	- - - - - - - R -	- - - - - - - R -	- - - - - - R - -	- - - - - - - R -	- - - - - - - - -	- - - - - - R - -	- - - - - - - - -
Eupenicillium hirayamae D. B. Scott & Stolk	-	R - - R R - - - -	- - - - - R - - -	- - - - - - - - -	- - - - - R R R -	- - - - R - - - -	- - - - - - - R -	- - - - - - - - -
Eupenicillium sp.	-	- - - - - - - - -	- - - - - - - R -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
Others								
Curvularia lunata (Wakker) Boedijn.	R	- - - - - - - - -	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- R - - - - - - -	R - - - - - - - -	- - - - - - - - -
Dreschlera sp.	R	R R - R - - - - -	R M - - - - - - -	- - - - - - - - -	R - - - - - - - -	M R - - - - - - -	R R - - - - - - -	R - R - - - - - -
Monascus mucoroides Van Tiegh.	-	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	R - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
Moniliella sp.	R	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -	- - - - - - - - -
Trichoderma sp.	-	- R - - - R - - -	- R - - - - - - -	- - - - - - - R -	- R - - - - - - -	R - R - - - - - -	- - - - - - - - -	- - - - - - - - -
Total no. of species detected	**14**	**23**	**27**	**21**	**28**	**28**	**24**	**21**

Keys : V : Very frequent occurring
M : Moderately frequent occurring
R : Rarely frequent occurring

** : Dominant species ; occurring at > 5 sampling times at all A_w levels.
* : Important species ; occurring at 3 - 5 sampling times at all A_w levels.
no asterisk : Minor species; occurring at ≤ 2 sampling times at all A_w levels.

citreonigrum, P. citrinum, P. griseoroseum, P. islandicum, P. minioluteum, P. purpurogenum, P. simplicissimum, P. spinulosum, P. thomii, P. variabile, P. verrucosum, P. vinaceum, Eupenicillium hirayamae, Eupenicillium sp., *Monascus mucoroides* and *Trichoderma* sp. Hence, the total number of species detected after storage at higher water activity levels was significantly more than the number of species detected initially.

The percentage incidence of dominant species occurring at each level of water activity at 25 °C was calculated as the percentage of the number of plates fungal species occurred per total number of plates at each time intervals based on the data in Appendix D(4.1). Hence, a summary is given in Appendix D(4.2) and illustrated graphically in Figs. 4.1- 4.6.

From Fig. 4.1, on ordinary rice, the water activity levels whereby there was a significant increase in the incidence from day 0 by *A. candidus* occurred at 0.65, 0.80, 0.85 and 0.98, by *A. flavus* at 0. 75 A_w and by *R. arrhizus* at 0.75 and 0.90 A_w. From Fig. 4.2, on glutinous rice, the water activity levels whereby there was a significant increase in the incidence from day 0 by *A. flavus* occurred at 0.7 and by *R. microsporus* at all water activities except 0.85. From Fig. 4.3, on riceflour, a significant increase in the incidence from day 0 by *A. flavus* and *R. arrhizus* occurred at water activity levels of 0.65 - 0.90, by *A. niger* at 0.80 and 0.90, by *A. terreus* and *P. chrysogenum* at all water activity levels and by *R. microsporus* at 0.75 and 0.85 A_w. From Fig. 4.4, on glutinous riceflour, a significant increase in the incidence of *A. flavus* occurred at all water activity levels except 0.95, by *A. niger* at all water activities, by *P. chrysogenum* at 0.90 - 0.98, by *R. arrhizus* at 0.80 and 0.85 and by *R. microsporus* at 0.90 A_w. On

Fig. 4.1 Changes in incidence of dominant species of fungi on ordinary rice stored at different levels of water activity at 25 °C

Incidence represented as a percentage of the number of plates species occurred per total number of plates over a storage period of 96 days.

Key : A.c., *Aspergillus candidus*; A.fl., *A. flavus*; A.n., *A. niger*; R.a., *Rhizopus arrhizus*; R.m., *R. microsporus.*

Fig. 4.2 Changes in incidence of dominant species of fungi on glutinous rice stored at different levels of water activity at 25 °C

Incidence represented as a percentage of the number of plates species occurred per total number of plates over a storage period of 96 days.

Key : A.fl., *A. flavus*; A.n., *A. niger*; R.a., *Rhizopus arrhizus*; R.m., *R. microsporus.*

Fig. 4.1 Changes in incidence of dominant species of fungi on ordinary rice stored at different levels of water activity at 25°C

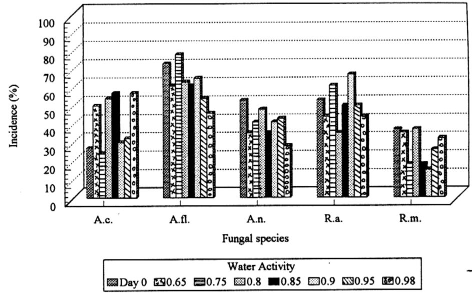


Fig. 4.2 Changes in incidence of dominant species of fungi on glutinous rice stored at different levels of water activity at 25°C

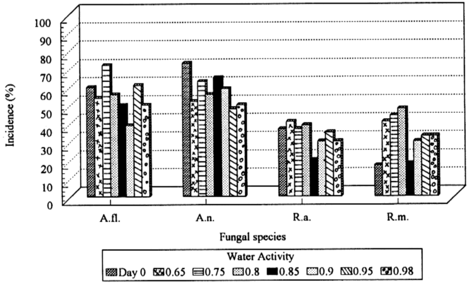


Fig. 4.3 Changes in incidence of dominant species of fungi on riceflour stored at different levels of water activity at 25 °C

Incidence represented as a percentage of the number of plates species occurred per total number of plates over a storage period of 96 days.

Key : A.fl., *A. flavus*; A.n., *A. niger*; A.ter., *A. terreus*; P.ch., *Penicillium chrysogenum*; R.a., *Rhizopus arrhizus*; R.m., *R. microsporus*.

Fig. 4.4 Changes in incidence of dominant species of fungi on glutinous riceflour stored at different levels of water activity at 25 °C

Incidence represented as a percentage of the number of plates species occurred per total number of plates over a storage period of 96 days.

Key : A.fl., *A. flavus*; A.n., *A. niger*; P.ch., *Penicillium chrysogenum*; R.a., *Rhizopus arrhizus*; R.m., *R. microsporus*.

Fig. 4.3 Changes in incidence of dominant species of fungi on riceflour stored at different levels of water activity at 25°C

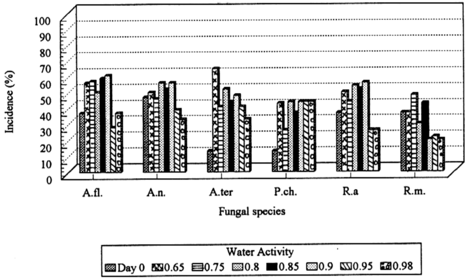


Fig. 4.4 Changes in incidence of dominant species of fungi on glutinous riceflour stored at different levels of water activity at 25°C

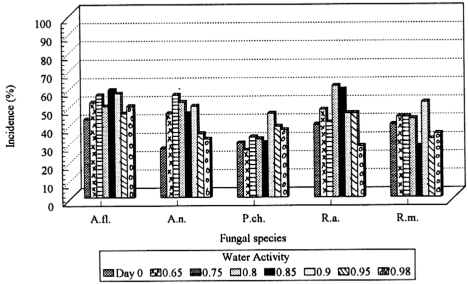


Fig. 4.5 Changes in incidence of dominant species of fungi on wheatflour stored at different levels of water activity at 25 °C

Incidence represented as a percentage of the number of plates species occurred per total number of plates over a storage period of 96 days.

Fig. 4.6 Changes in incidence of dominant species of fungi on cornflour stored at different levels of water activity at 25 °C

Incidence represented as a percentage of the number of plates species occurred per total number of plates over a storage period of 96 days.

Fig. 4.5 Changes in incidence of dominant species of fungi on wheatflour stored at different levels of water activity at 25°C

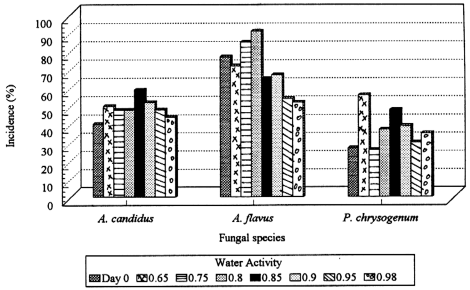
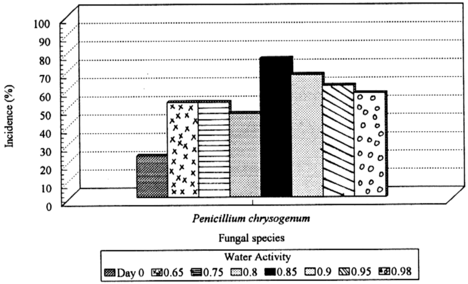


Fig. 4.6 Changes in incidence of dominant species of fungi on cornflour stored at different levels of water activity at 25°C



wheatflour (Fig. 4.5), a significant increase in the incidence from day 0 by *A. candidus* occurred at all water activity levels, by *A. flavus* at 0.75 and 0.80 and by *P. chrysogenum* at 0.65, 0.80, 0.85, 0.90 and 0.98. On cornflour (Fig. 4.6), a significant increase in the incidence from day 0 by *P. chrysogenum* occurred at all water activity levels. Hence, the dominant species showed a variation in water activity levels at which its frequency is highest depending on the starch-based food.

The growth rates of dominant and important fungal species isolated from starch-based food are presented in Appendix D(4.3) and the average displayed in Table 4.8. *Rhizopus arrhizus* was very fast growing covering the petri dish in 2 days followed by *R. microsporus* with growth rate of 31.8 mmday^{-1} diameter. The moderately fast growing species were *Moniliella* sp. (11.5 mmday^{-1} diameter), *A. flavus* (9.4 mmday^{-1} diameter), *A. fumigatus* (9.0 mmday^{-1} diameter), *A. niger* (8.2 mmday^{-1} diameter), *Absidia corymbifera* (8.2 mmday^{-1} diameter) and *A.clavatus* (5.9 mmday^{-1} diameter) in that order. The slow growing species were *A. candidus* (4.3 mmday^{-1} diameter), followed by *P. chrysogenum* (3.5 mmday^{-1} diameter), *P. islandicum* (2.6 mmday^{-1} diameter) and *A. terreus* (2.4 mmday^{-1} diameter).

The amylolytic activity exhibited by each fungal species were categorized as strongly amylolytic i.e when the zone void of blue is equal to or up to 5 mm less than the colony diameter; moderately amylolytic i.e when the zone is more than 5 mm less than the colony diameter and non-amylolytic in the absence of the zone void of blue. Hence, as shown in Table 4.8, *P. islandicum, A. terreus, P. chrysogenum* and *A. fumigatus* were strongly amylolytic (Plate 4.1) and *A. flavus, Moniliella* sp., *A. clavatus* and *A. candidus* were moderately amylolytic (Plate

4.2). *Rhizopus arrhizus, R. microsporus, A. niger* and *Absidia corymbifera* were found to be non-amylolytic (Plate 4.3).

After 7 days of growth on starch-extract agar at 25 °C, the fungal induced changes brought about by each fungal species were an increased in the presence of maltose as a result of the breakdown of starch and a reduction in pH as compared to the control (Table 4.8).

Table 4.8 Fungal growth rates, amylolytic activity and changes they induced on starch-extract agar after 7 days of growth at 25 °C

Fungal species	Growth rate * (mm per day) diameter ± S. E	Amylolytic # activity	Fungal induced changes	
			maltose # ($mgml^{-1}$) ± S.E.	pH
Aspergillus candidus	4.3 ± 1.2	moderate	0.99 ± 0.07	6.2
A. clavatus	5.9 ± 0.4	moderate	0.94 ± 0.12	5.0
A. flavus	9.4 ± 0.6	moderate	*A. niger* 2.03 ± 0.25	5.9
A. fumigatus	9.0 ± 0.5	strong	0.90 ± 0.08	5.4
A. niger	8.2 ± 0.4	non	0.92 ± 0.05	4.3
A. terreus	2.4 ± 0.5	strong	0.81 ± 0.05	6.1
Penicillium chrysogenum	3.5 ± 0.8	strong	0.68 ± 0.02	5.7
P. islandicum	2.6 ± 0.2	strong	0.63 ± 0.04	5.3
Absidia corymbifera	8.2 ± 0.8	non	0.63 ± 0.21	6.0
Rhizopus arrhizus	n.d.	non	2.40 ± 0.31	4.4
R. microsporus	31.8 ± 1.4	non	1.56 ± 0.08	5.6
Moniliella sp.	11.5 ± 1.2	moderate	1.95 ± 0.05	4.1
Control	-	non	0	6.2

* Average of 6 determinations

Average of 3 determinations

n.d. : insufficient data to calculate growth rate as *A. rhizopus* is very fast growing covering the petri dish by 2 days.

Plate 4.1 Strongly amylolytic fungi isolated from starch-based food

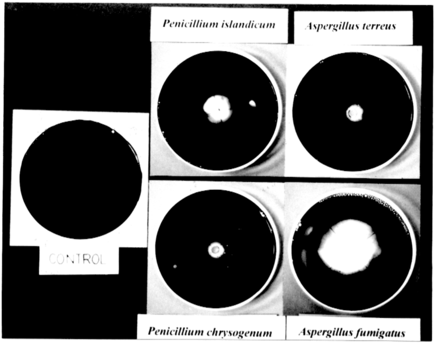


Plate 4.2 Moderately amylolytic fungi isolated from starch-based food

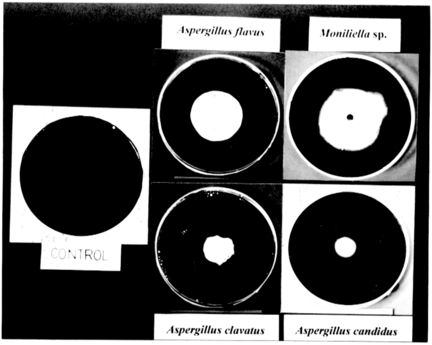
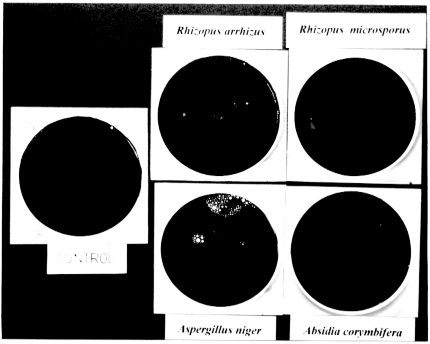


Plate 4.3 Non-amylolytic fungi isolated from starch-based food



4.4 DISCUSSION

Water is an important factor limiting or prolonging the rate of colonisation of stored commodity by fungi. At optimum temperature for growth, its availability determines whether a fungal spore can germinate and how quickly. It will also determine the rate of growth of different species and their ability to compete with one another. A range of interspecific interactions, ranging from mutual intermingling, through mutual inhibition or combat to dominance by individual species have been recognised as influencing the community structure on a substrate (Cooke and Rayner, 1984).

Grains and its resultant flour contained a varied microflora of bacteria, yeasts and filamentous fungi. Fungi are present as spores or mycelium. Starch-based food even though of low moisture content are rich substrate and, under conducive environmental conditions, a wide range of fungi will develop. In this study, storage of starch-based food at different levels of water activity for 96 days resulted in the isolation of 13 species from the genera *Aspergillus* and its teleomorph, 22 species from the genera *Penicillium* and its teleomorph, 6 Zygomycetes and 5 other species. The list and descriptions to the species isolated are given in Chapter 5 and the key to its identification is given in Chapter 6. A quick perusal of Tables 4.2 - 4.7 indicate that the most characteristic genera of the storage environment are *Aspergillus* and *Penicillium* species. The number of dominant *Aspergillus* species capable of causing spoilage is more than *Penicillium* species of which only *P. chrysogenum* is dominant. This is because the starch-based food investigated are foods of reduced A_w often referred to as intermediate moisture foods. *Penicillium* species usually require water to be more readily available than *Aspergillus* species for growth. According to Christensen (1970; 1978), fungi from the genus *Aspergillus* can grow and spread on grains at moisture content < 17% while Penicillia at moisture content > 17%. The moisture contents of the starch-based foods studied was stored at moisture contents ranging from 9.30 - 21.93% for ordinary rice; 10.14 - 25.57% for glutinous rice; 6.76 - 20.87% for riceflour; 5.75 - 23.63% for glutinous riceflour; 6.67 - 23.11% for wheatflour and 6.39 - 20.69% for cornflour depending on the water activity levels. The water activity corresponding to 17% moisture content of starch-based food is approximately 0.90 and *Penicillium* spores if present or still viable should grow when conditioned to ≥ 0.9 A_w since at optimal temperature and humidity levels germination of conidial spores usually starts promptly (Snow, 1949).

Amongst the Aspergilli, the species found ranges from the extremely xerophilic (< 0.75 A_W) i.e. *Eurotium repens*; the moderately xerophilic (0.75 -

0.79 A_w) i.e. *A. versicolor, A. sydowi, A. terreus, A candidus, A. flavus, A. tamarii, A. niger, A. aculeatus* and *A. clavatus*; to the slightly xerophilic (0.80 - 0.89 A_w) i.e. *A. fumigatus. Aspergillus flavus* was dominating on all six starch-based foods stored for 96 days at all A_w with a moderate or high frequency within 0.75 - 0.80 A_w. *Aspergillus flavus* required a minimum A_w of 0.78 at 33 °C for growth as reported by Ayerst (1969) and Smith and Onions (1994). *Eurotium repens* being an uncompetitive species at 15 - 30 °C was rarely frequent at all A_w although initially they are present rarely on riceflour and cornflour and moderately frequent on ordinary rice, glutinous rice, glutinous riceflour and wheatflour.

The importance attached to *A. flavus* being dominant on most starch-based food investigated reflects the risk of aflatoxins being produced in food when improperly stored. Cyclopiazonic acid may also be produced by *A. flavus* and have a role in toxicity. In addition, other toxin-producing Aspergilli which are dominating are *A. candidus, A. niger* and *A. terreus*. These species has been shown to produce mycotoxins in foods. Consuming small doses of mycotoxins over a long period may lead to poor food conversion, infertility and with some mycotoxins, to the development of cancer.

Amongst the Penicillia, *P. chrysogenum* was dominating on all flour samples throughout storage at 0.65 - 0.98 A_w. The spores are still viable in the dried starch-based food and *P. chrysogenum* required a minimum A_w of 0.79 for growth as reported by Armolik and Dickson (1956). It should be noted that the occurrence of *P. islandicum* on ordinary rice, glutinous rice, riceflour and glutinous riceflour was quite regular at all water activity levels. This species has long been believed to have a major habitat in rice, but Pitt (1995) quoted that recent investigations have cast doubt on this. Hence, this study contradicts Pitt's suggestion. The importance relating to this species is that it has been known to produce at least four mycotoxins: islanditoxin, cyclochlorotine, luteoskyrin, and erythroskyrin. The other Penicillia required a higher A_w of 0.80 - 0.90 did not survive long-term storage in the absence of favourable growth conditions. According to Lacey and Magan (1991), at favourable temperature for fungal growth, if the moisture content is not high enough to grow, *Penicillium* spp. will remain low or declined throughout storage. Hence, the infrequent occurrence on the initial composite samples (at day 0) indicate that all the starch-based food sampled from the retail outlets has been stored appropriately dried.

Amongst the hydrophilic zygomycetes, *R. arrhizus* is most dominant followed by *R. microsporus* and *Absidia corymbifera*. This indicate that their spores can survive at ambient temperature throughout storage at low moisture content. When exposed to high A_w rapid germination took place. Although there are occasional reports of the ability to produce toxic metabolites there is no strong evidence to implicate this group of fungi in mycotoxicoses (Smith and Moss, 1985). However their frequent occurrence is important as one of the agents of food spoilage and *Rhizopus* have been found to affect germination of fungi (Misra *et al.* 1969).

As expected, the presence of field fungi on all six starch-based food at all A_w were insignificant as they require a high moisture content to grow. Moisture contents of 22 - 25% (wet wt. basis) or of 28 - 33% (dry wt. basis), have been cited by Christensen (1965) and Christensen and Kaufman (1965) as the lower limit for growth and development for field fungi. The relatively high moisture content requirements have been explained by several authors as the reason for the relative infrequency of active proliferation of field fungi in storage, as oppose to mere persistence, which can also be seen from this study, can still take place under dry conditions.

Amongst the six starch-based foods, contamination by fungi is least in cornflour stored at all A_w. In the other starch-based foods, proliferation of fungi at the lowest A_W (0.65) did occur at 25 °C. Hence, water activity lower than 0.65 is required to prevent fungal spoilage of starch-based food for long term storage.

Rhizopus arrhizus, *R. microsporus*, *Absidia corymbifera* and *A. niger* were capable of growing at very high growth rates on starch-extract agar even though they were found to be non-amylolytic. The importance attached to this is that the high metabolic activities of these species can generate heat and moisture and hence initiate the development of other fungi including toxin-producing species. This successions of fungi have been observed by Flannigan (1977) in stored grain and by Clarke *et al.* (1968) in cereal bulks stored for animal feed. The reduction in pH after 7 days of fungal growth was attributed to the biodeterioration of starch to its end-products. Hence, the high growth rates together with the amylolytic activity exhibited by the dominant and important fungal species growing on starch-based food induces significant levels of changes in the starch-based food indicate their participatory roles in the spoilage of starch-based food. Of greater significance is the frequent implication of many of those species in the production of highly potent toxins (Richard, 1990; Gedek, 1994).