CHAPTER 1.0 GENERAL INTRODUCTION

1.1 BASIC CONCEPTS OF WATER AVAILABILITY IN FOOD

Water availability for microbial growth can be measured by equilibrium relative humidity (ERH), water potential (ψ) and water activity (A_w). Equilibrium relative humidity is the relative humidity of the intergranular air in equilibrium with the water content of the product and ψ is the free energy of water in a system relative to the free energy of a reference pool of pure, free water of specified mass or volume which is considered to have zero water potential. The term water activity, or A_w, has been found to be a useful means of characterizing the state or condition of water in foods by food microbiologists and food scientists.

1.1.1 Water Activity (A_w)

The definition of A_w most frequently cited is based on Raoults's Law wherein the ratio of the vapour pressure of water in a product or solution to that of pure water at the same temperature and pressure. By definition, (Scott, 1957), A_w is also equal to the equilibrium relative humidity (ERH) divided by 100 i.e. ERH and A_w are numerically equivalent but ERH is expressed as a percentage while A_w as a decimal fraction of one. Hence,

$$A_w = \frac{Vps}{Vpw} = \frac{\%ERH}{100}$$

where Vps is the vapour pressure of water in food, Vpw the vapour pressure of pure water at the same temperature and %ERH the relative humidity at which food neither gains nor loses moisture to the atmosphere. Since Vps varies with the product, then the percent gross moisture content of each foodstuff on which microbes grow will vary in terms of A_w. At A_w 1.00 free water is available in the substrate.

1.1.2 Sorption effects

Water present in foods consist of bound and unbound water. The former relates to the fact that the water molecule forms intermolecular hydrogen bonds and can therefore be bonded to the functional groups of proteins and carbohydrates. The Brunauer-Emmett-Teller (BET) theory is the most superior method to characterize the adsorption of water in many materials, including foods. In essence, the BET absorption theory predicted the presence of a monomolecular layer of unfreezable water which could inhibit interactions between adjacent polar groups in food, and exclude oxygen adsorption and a number of other functions. Considering the contrapositional, unbound water, a vast number of desirable and undesirable reactions occurred in the unbound water within foods including, at a variety of elevated unbound water quantities, microbial growth.

A plot of A_w value as a function of the amount of water present is termed a sorption isotherm (Fig. 1.1). It reflects the manner in which water is bound (or unbound). **Region A** of this isotherm corresponds to the monolayer or monomolecular film, which is bound to ionic entities such as amino and carboxyl groups. Within this region, water is generally unavailable for reaction (Labuza, 1975). Isotherm **region B** specifies accumulation of additional layers of water and is, in fact, a transition zone between region A and **region C**, the latter representing a zone of free water existing primarily in capillaries. In this region water is freely available for the life functions of microorganisms. As a result, greater varieties of microorganisms proliferate as the A_w value is increased from about 0.60 upwards.

Theoretically, the sorption isotherms shown in Fig 1.1 is a single sigmoid curve, the shapes depending on whether water is removed (desorption) from a

higher moisture food or water is added (adsorption) to a food originally of lower moisture content. This results in the hysteresis loop which, as equilibrium approaches, collapses in the directions shown by the arrows to a thermodynamically stable entity. The shape is strongly temperature dependent and can vary widely, depending on the composition of the food.

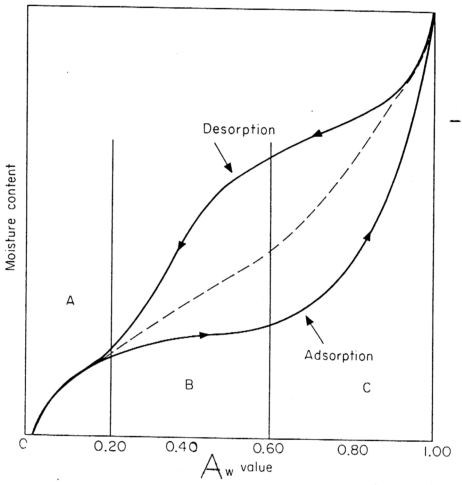


Fig. 1.1 **Relationship between moisture content and A_w value for foods showing typical hysteresis loop consisting of adsorption and desorption isotherms**

(The theoretical isotherm at equilibrium is shown by the dotted line)

1.2 FACTORS AFFECTING FUNGAL GROWTH AND MYCOTOXIN PRODUCTION IN FOOD

In stored food, physical factors dictate fungal growth and mycotoxin formation. There are eight physical parameters which affect the growth of fungi in food : temperature; water activity; pH; oxygen (and CO_2) tension; type of substrate and nutritional factors; consistency i.e. solid or liquid state; chemical treatment and the presence of preservatives; and specific solute effects. Each of these factors is important in some aspect of food preservation. However, in bulk, unprocessed foods (commodities), the most important factors affecting fungal growth are water activity and temperature.

1.2.1 Water activity

The tolerance of low A_w by different classes of fungi is often sharply defined. Certain storage fungi which occur in dried foods have the ability to grow at lower A_w levels than other organisms. Most species of fungi require at least 0.75 A_w while no fungal growth occur at A_w levels below 0.60 (Pitt & Christian, 1968; Lacey *et al.*, 1980). *Xeromyces bisporus* Fraser (= *Monascus bisporus* (Fraser) V. Arx) is the most xerophilic fungus known, with a minimum A_w for growth of only 0.61. As A_w increases above this level, an increasing number of fungi can grow and metabolic activity increases until it becomes sufficient to cause spontaneous heating. With A_w levels of 0.7 - 0.8, the fungal species that may grow include *Aspergillus*, *Penicillium* and *Eurotium*, which represent the typical contaminating fungal genera in stored grain (Manabe and Tsuruta, 1991). Some mycotoxigenic fungi, notably *Aspergillus flavus* Link., *A. parasiticus* Speare and *A. ochraceus* Wilhelm are favoured by a combination of low A_w and relatively high

storage temperatures, and are thus more likely to be found in products stored under tropical conditions. *Fusarium* species are field fungi having very high water requirements, hence, *Fusarium* mycotoxins are not produced during storage. *Penicillium* species have water requirements intermediate between those of *Aspergillus* and *Fusarium* species. So far as is known most *Penicillium* species require high water activities for germination, growth and mycotoxin production (Pitt, 1975; Hocking and Pitt, 1979).

1.2.2 Temperature

Fungi differ widely in the ranges of temperature that allow their growth and in that giving optimum growth. There are three classes of fungi based on the temperature range which is optimum for growth. Most fungi are mesophiles; i.e. growing best between 10 - 40 °C. The psychrophiles have an optimum temperature between 5 - 10 °C and cryophiles are able to grow at very low temperatures. The thermophiles can grow at temperatures above 40 °C and some even higher than 50 °C. Most fungi found in stored products thrive within the range 10 - 40 °C and have optima of 25 - 35 °C. According to Christensen and Kaufman (1965), storage temperature of 30 °C are optimal for spoilage, which is slow at 12 - 15 °C and almost ceases at 5 - 8 °C. Lowering temperature decreases the rate of metabolism and is often used to slow deterioration.

Fungal spores are less resistant to heat. The most resistant fungi are ascomycetes, in which the ascospores are far more resistant than the conidia, with *Byssochlamys* spp. and *Neosartorya fischeri* (Wehmer) Malloch and Cain able to survive 10 min heating at 90 °C.

The effect of temperature is seldom important in the tropics. Almost all commodities are stored at ambient temperatures (25 - 30 °C), which are entirely favourable for fungal growth and mycotoxin production. In warmer climates, water activity is thus the dominant factor affecting fungal growth and mycotoxin production after harvest and during storage.

1.2.3 Interaction of temperature and water activity

Interaction between A_w and temperature largely determine the range of individual fungi which can germinate, grow and sporulate and, therefore, has a large influence on their role in the stored food ecosystem. Changing either factor affects the growth rate and may affect the ability of a fungus to compete with other species. The conditions under which fungi are most numerous are not necessarily those at which they grow best.

Greatest tolerance of low A_w usually occurs close to the optimum temperature for growth but the minimum A_w tolerated may be modified by pH and solute in experimental systems. The conditions for growth and mycotoxin production of *Aspergillus flavus* have been thoroughly studied. The optimum temperature for growth of *A. flavus* is around 33 °C, with growth limits between 12 °C and 43 °C at an optimum A_w of 0.98 - 0.99 (Ayerst, 1969; Pitt and Hocking, 1977). It is a xerophile according to the definition of Pitt (1975) but its lower limit for growth can vary according to the medium used and the temperature of incubation. Pitt and Hocking (1977) reported the minimum A_w for growth of *A. flavus* at 0.83 at 25 °C, while Ayerst (1969) found this fungus capable of growth at 0.78 A_w at 30 - 33 °C.

Aflatoxin production by *A. parasiticus* is similarly affected by temperature and A_w. Northolt *et al.* (1976), working with *A. parasiticus*, showed that the most favourable conditions for aflatoxin production were similar to those for optimum growth, i.e. temperature ranging from 25 - 32 °C at 0.99 A_w. At 0.94 A_w, however, the optimum temperature for toxin production was 24 °C. The lowest A_w at which toxin was detected was 0.88 and the lowest temperature was 13 °C. The minimum A_w for growth of *A. parasiticus* was reported by these workers to be 0.82. They showed that reduction of both temperature and A_w can prevent the elaboration of aflatoxins by *A. parasiticus.*

A_W and temperature effects on mycotoxin production often differ for two toxins produced by the same species and for a toxin produced by two different species. For instance, ochratoxin and penicillic acid production by *A. ochraceus* on poultry feed was greatest at 30 °C and 0.95 A_w and at 22 °C and 0.90 A_w respectively (Bacon *et al.*, 1973). The minimum A_w allowing growth of 11 species of fungi was usually lower and the temperature range wider than those permitting aflatoxin, patulin, penicillic acid or ochratoxin production (Northolt and Bullerman, 1982). However, the minimum A_w permitting formation of ochratoxin and penicillic acid by *Penicillium aurantiogriseum* Dierckx were about 0.90 A_w and 0.97 A_w respectively, while those for the same toxins produced by *A. ochraceus* were about 0.85 A_w and 0.88 A_w (Bacon *et al.*, 1973; Northolt and Bullerman, 1982). Production of tenuozonic acid by *Alternaria tenuissima* (Kunze ex Pers.)Wilts. on cotton seed was optimum at 20 °C and 1.00 A_w, halved at 0.95 A_w and prevented at 0.85 A_w (Young *et al.*, 1980).

1.2.4 pH

The hydrogen ion concentration affects the ionic state and therefore the availability of many metabolites and inorganic ions to an organism, hence its influence on the state and activity of many cellular components cannot be over emphasized. Fungi are tolerant of a wide range of pH, most species growing between pH 2 and 8.

1.2.5 Oxygen (and carbon dioxide) tension

The majority of fungi are aerobic and need a minimum amount of atmospheric oxygen for growth and efficient mycotoxin production. With many fungi, the concentration of oxygen in the atmosphere must be decreased to less than 0.14% before linear growth is decreased by 50%. Increases in CO_2 concentration to 5 - 10% can stimulate growth of some species, especially when A_w is high, and > 15% CO_2 may be required to halve growth rates.

Fungi differ in their tolerance of low O_2 and high CO_2 concentrations and species may be more greatly affected when both components are altered rather than either alone. Thus, they may respond more to high CO_2 when O_2 concentration is low than when it is close to ambient conditions.

Atmospheres high in CO_2 are more effective in controlling fungal growth than those which exclude O_2 by replacement with nitrogen (Hocking, 1991). In addition, two reports showed an inhibition of aflatoxin production when the CO_2 was increased to 20% - 40% at 17 °C (Sanders *et al.*,1968) and at 40% CO_2 and 5% O_2 (Pande and Saxena, 1990). Most fungi were not killed by high CO_2 concentration and the biosynthetic pathways for mycotoxin production were only blocked but not damaged. When restored to air, growth was resumed and

mycotoxins were produced to the usual extent (Paster, 1987). - Modified atmospheres have been used for the control of both insects and fungi in stored grains but nitrogen must be oxygen free while CO_2/O_2 regimes suitable for controlling insects are insufficient to control fungi.

1.2.6 Type of substrate and nutritional factors

Although the pattern of colonization of different substrates is similar, the exact nutritional requirements for each species differ from all others and thus, for a given substrate, only those species occur for the growth of which the substrate is suitable. Furthermore the nutritional requirements for fungal growth and those for mycotoxin production may be different i.e. there may be specific differences for substrate rich in oil, protein or starch. For example, *A. flavus* preferably occur and produces toxin in oilseeds than in other grains. Among twelve natural substrates studied, oats and hay were found to be most favourable for gliotoxin synthesis by *A. fumigatus* (Kurbatskaya and Trostanetskii, 1987).

Penicillium urticae Bainier and *P. patulum* Bainier on Czapek-Dox media require glucose or maltose for maximum patulin production (Yamamoto, 1954). *Penicillium purpurogenum* Stoll require malt and yeast extracts for rubratoxin biosynthesis (Natori *et al.*, 1970), but rubratoxin production by *P. rubrum* Stoll was inhibited by yeast extract even though stimulated by malt extract (Hayes and Wilson, 1968; Townsend *et al.*, 1966).

Aspergillus flavus prefers glucose, sucrose or fructose for maximum aflatoxin production (Mateles and Adye, 1965). Mannose and xylose increased aflatoxin production by *A. parasiticus* however, inhibited aflatoxin production by *A. flavus.* Corn steep liquor (Schroeder, 1966), yeast extracts, peptone and casein

hydrolyzate stimulate aflatoxin production by *A. parasiticus* or *A. flavus* (Mateles and Adye,1965; Davis *et al.*, 1966, 1967; Davis and Diener, 1968) and glycine, glutamic acid and to a moderate degree, alanine, aspartic acid and glutamine stimulate aflatoxin production by *A. flavus* (Davis *et al.*, 1967). Optimal ochratoxin biosynthesis by *A. ochraceus* has been observed to require media containing 3% sucrose or 1 to 2% sucrose combination compared to galactose, which generated only 75% of the yield of aflatoxin (Fereira, 1966).

Fungi, as with other living organisms require trace elements for growth and mycotoxin production. For example, zinc and iron are required for aflatoxin production, but iron inhibits mycelial growth (Nesbitt *et al.*, 1962; Davis *et al.*, 1967). The production of aflatoxin has been reported to be stimulated by inorganic nitrogen sources such as $(NH_4)_2SO_4$ and KNO_3 (Eldridge, 1964; Mateles and Adye, 1965). Molybdenum also stimulates aflatoxin production (Davis *et al.*, 1967). Ochratoxin production by *A. ochraceus* NRRL 3174 required zinc, iron and copper. Either an excess or a deficiency of these elements suppresses mycotoxin production as well as fungal growth (Steele *et al.*, 1973).

1.2.7 Consistency

Consistency exerts considerable influence over the kind of spoilage a food will undergo. Generally speaking, yeasts cause more obvious spoilage in liquid products, because single celled microorganisms are able to disperse more readily in liquids. In contrast, filamentous fungi are assisted by a firm substrate. Even so fungi can also spoil liquid products as yeasts can spoil solid products.

1.2.8 Chemical treatment and the presence of preservatives

An increasing number of biocides have now been studied for their effect on fungal growth and mycotoxin production. Infection of fruit and vegetables during storage may be prevented or decreased by the use of fungicides before harvest or as dips before storage and growth of moulds in foods and stored products may be controlled by using weak acids such as benzoic, sorbic, propionic, acetic, nitrous or sulphurous acids.

Treatment of cereal grains with fumigants such as phosphine and other chemicals to control insects and other pests may affect the production of mycotoxins. The effect of prior treatment of wheat with phosphine increases aflatoxin B_1 production by *A. flavus* from 10 to 37% depending on the strain. This treatment reduced aflatoxin production by *A. parasiticus* by 12 to 20%, again depending on the strain. Ochratoxin production by an *A. ochraceus* strain was increased by 17% but decreased by 3% with *Penicillium viridicatum* Westling. With the CCL_4-$CHCL_3$ mixture (80 : 20, w/w), production of these mycotoxins by corresponding fungal species and strains was increased; the percentage increases ranged from 5 to 113% for aflatoxin for both *A. flavus* and *A. parasiticus*. The percent increase for ochratoxin was around 3% for *A. ochraceus* and 257 % for *P. viridicatum* (Vandergraft *et al.*, 1973).

Although insecticides have some antifungal effects, it is probable that their main activity in the presence of biologically active fungal mycelium is to interfere with some reaction which is essential for the pathways of secondary metabolism and it does seem that, in general, these agents reduce the specific production of particular mycotoxins. In contrast, antifungal agents would be expected to have a

much more profound effect on the primary metabolism of fungi and, under such conditions of stress, they may occasionally be expected to enhance secondary metabolism, including the production of mycotoxins.

1.2.9 Specific solute effects

Solutes present in foods can exert additional effects on the growth of fungi. Scott (1957) reported that *Eurotium amstelodami* Mangin grew 50% faster at its optimal A_w (0.96) when A_w was controlled by glucose rather than magnesium chloride, sodium chloride or glycerol. Pitt and Hocking (1977) showed a similar effect for *Eurotium chevalieri* Mangin, and reported that the extreme xerophiles *Chrysosporium fastidium* Pitt and *Xeromyces(Monascus) bisporus* grew poorly if at all in media containing sodium chloride as the major solute. In contrast Pitt & Hocking (1977) and Hocking and Pitt (1979) showed that germination and growth of several species of *Aspergillus* and *Penicillium* was little affected when A_w of the media was controlled with glucose-fructose, glycerol or sodium chloride.

1.3 MYCOTOXINS

1.3.1 Definition

Mycotoxins are a group of secondary metabolite of certain strain of a number of species of fungi which may cause pathological or undesirable physiological responses in man and other animals (Smith and Moss, 1985). Mycotoxins are produced on a wide range of substrates under a diverse range of conditions.

1.3.2 History of mycotoxins

Prior to 1960, diseases caused by mycotoxins were always of an ill-defined nature. There was little interest in mycotoxin research, although, in retrospect the connection between the consumption of mouldy food products and certain types of human illness was recognized in the middle ages, as in the case of ergotism (Ainsworth, 1976). In addition, a serious, epidemic human mycotoxicoses known as alimentary toxic aleukia (ATA) occurred in the Soviet Union in the 1940s or earlier, and Russian scientists had recognized the relationship between the consumption of mouldy, overwintered cereal grains and the disease (Joffe, 1965). In Japan, scientists also found an association between certain disease syndromes and the consumption of mouldy rice (Tsunoda, 1970). In the United States, investigators in the south also recognized the association between certain animal mycotoxicoses and the consumption of mouldy corn (Burnside *et al.*, 1957). However, all these observations did not arouse great interest until the 1960s following the report of the "Turkey X" disease . The accident resulted from aflatoxin-contaminated Brazilian peanut meal in feedstuffs which killed more than 100,000 young turkeys and ducklings. At about the same time, liver cancer was observed in fish hatchery trout in the U.S.A. This outbreak of cancer was related to aflatoxin-contaminated cottonseed meal in the fish feed (Halver, 1969). Great strides in mycotoxicology were accomplished following the report and still continue to occur.

Later it was learned that aflatoxin B_1, the most potent carcinogen for experimental animals found in the world (WHO, 1979) may be related to human hepatocancer (Keen and Martin, 1971; Shank *et al.*, 1972). After 30 years, the

human carcinogenecity of aflatoxin has again been confirmed by the International Agency for Research on Cancer (IARC) evaluating naturally occurring mixtures of aflatoxins as class 1 human carcinogens (IARC, 1993).

In the 1970s and early 1980s, some man-made mycotoxin problems were reported from some Southeast Asian countries These incidents were reportedly caused by some trichothecene mycotoxins, produced by *Fusarium* species (Goto, 1990). Many scientists began to pay attention to trichothecene mycotoxins, such as deoxynivalenol, nivalenol and T-2 toxin. Through studies, the natural occurrence of deoxynivalenol or some other trichothecene mycotoxins in some agricultural products in the U.S.A., Canada and Netherlands became known (Vesonder *et al.*, 1978; Scott *et al.*, 1980; Snijders, 1990).

Currently, at least 3000 metabolites are produced by fungi of which 300 or so mycotoxins are known to be produced by several genera of mycotoxin-producing fungi (Cole and Cox, 1981).

1.3.3 Structure and formation of mycotoxins

Mycotoxins are non-antigenic and of low molecular weight complex organic compounds. Being a secondary metabolite it is difficult to catalogue and classify mycotoxins by their chemical structure alone, due to their enormous diversity in chemical structure. However, they can be classified in terms of biosynthetic pathways leading to their production. This is so because the processes of primary and secondary metabolism are linked by the same pool of important precursor molecules required for the biosynthesis of primary metabolites associated with growth (Fig.1.2). It frequently happens that a particular secondary metabolite is itself a precursor for a series of reactions leading to the formation of

further groups of compounds. It is commonly observed that the more steps there are leading to the formation of a mycotoxin, the more limited is the number of species producing it.

Primary metabolites	Precursor	Secondary metabolites
Fatty acids	--------> Acetyl coenzyme A ------>	Polyketides
Sterols	--------> Mevalonic acid ------>	Terpenes
Proteins	--------> Amino acids ------>	Cyclic polypeptides
Aromatic amino acids	--------> Shikimic acids ------>	Phenolic acids, Diphenylbenzoquinones

Fig. 1.2 Intermediates linking primary and secondary metabolism (Adapted from Smith and Moss, 1985)

In the polyketide pathway, acetyl coenzyme A and a number of molecules of malonyl coenzyme A are coupled together in the presence of carbon dioxide for the production of fatty acids. The ketone groups formed as each C_2 unit is added are reduced continuously to give the paraffin chain of the final product. If these ketone groups are not reduced, the resulting compound will be very reactive and can undergo a series of condensation reactions often leading to the formation of ring compounds. Such intermediates have been called polyketides and be classified as tri-, tetra-, pentaketides etc. depending on the number of acetyl groups incorporated. The relationship of the polyketide pathway to a number of mycotoxins is shown in Table 1.1.

Table 1.1 Polyketide-derived mycotoxins

(Adapted from Smith and Moss, 1985)

Group	Mycotoxins	Genera
Tetraketides	Patulin	*Penicillium, Aspergillus, Paecilomyces*
	Penicillic acid	*Penicillium*
Pentaketides	Citrinin	*Penicillium*
	Ochratoxin	*Aspergillus, Penicillium*
	Austdiol	*Aspergillus*
Hexaketides	Maltoryzine	*Aspergillus*
Heptaketides	Viomellein	*Aspergillus, Penicillium*
	Viopurpurin	*Trichophyton, Penicilium*
	Rubrosulphurin	*Aspergillus, Penicillium*
	Xanthomegnin	*Trichophyton, Aspergillus, Penicilium*
	Citromycetin	*Penicillium*
	Alternariol	*Alternaria*
	Altenuene	*Aternaria*
	Altenuic acid	*Alternaria*
Octaketides	Luteoskyrin	*Penicillium*
	Rugulosin	*Penicillium*
	Rubroskyrin	*Penicillium*
	Islandicin	*Penicillium*
	Secalonic acid	*Claviceps, Aspergillus, Phoma*
Nonaketides	Citreoviridin	*Penicillium, Aspergillus*
	Asteltoxin	*Aspergillus*
	Zearalenone	*Fusarium*
	Viridicatum toxin	*Penicillium*
Decaketides	Aflatoxin	*Aspergillus*
	Sterigmatocystin	*Aspergillus*
	Austocystin	*Aspergillus*

The pattern of folding and condensation of the initial polyketide chain is fairly specific for any particular strain of fungi and, presumaby, takes place on a specific enzyme surface. Diversification of mycotoxins may arise from a single polyketide chain by:

i. rearrangement of the initial condensation product e.g. patulin and penicillic acid,

ii. introduction of a different number of C_1 substituents into the pentaketide

skeleton in the biosynthesis of ochratoxin, austdiol and citrinin,

iii. a relatively simple folding pattern e.g. folding of a partially reduced nonaketide in the biosynthesis of zearalenone, and

iv. loss of C_2 unit during the formation of the bis-furan rings e.g. from a decaketide in the formation of aflatoxin.

In the mevalonate pathway, mevalonic acid undergoes pyrophosphorylation, decarboxylation and dehydration to form the active isopentenyl pyrophosphate precursor of such key compounds as sterols, steroids and carotenoids. This intermediate is also the starting point for the biosynthesis of a group of mycotoxin called trichothecenes.

The trichothecenes are a large family of toxins all having a complex sesquiterpene (C_{15} skeletons) nucleus in common derived from farnesyl pyrophosphate by a series of cyclizations and methyl group migrations. Examples of trichothecenes are given in Table 1.2.

Table 1.2 A selection of trichothecenes and their sources

(Adapted from Smith and Moss, 1985)

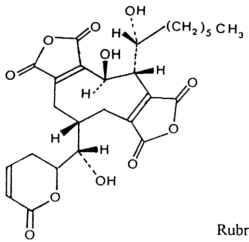
Trichothecin	Genera
Trichodermol	*Myrothecium*
Trichodermin	*Trichoderma*
Trichothecin	*Trichothecium*
Crotocin	*Cephalosporium*
T-2 toxin	*Fusarium*
Nivalenol	
Deoxynivalenol (vomitoxin)	
Fusarenon	

A number of mycotoxins are also produced by the incorporation of amino acids into either macrocyclic peptides such as malformin C and islanditoxin or

highly condensed polycyclic compounds such as sporidesmin, gliotoxin and ergotamine. Gliotoxin is formed from phenylalanine and serine, while sporidesmin is produced from tryptophan and alanin. The formation of both may proceed via an intermediate containing an epoxide ring in its structure.

Mycotoxins can also be derived from a mixture of two or more pools of biosynthetic precursors. For example, the linking of amino acids to a number of mevalonate derived isoprene units. These isoprene units may be simply peripheral substituents or they may be involved in the production of a polycyclic skeletons. Aflatrem (from *A. flavus*) shows examples of both situations while roquefortine (from *P. roquefortii* Thom) is a diketopiperazine derived from tryptophan and histidine, substituted in the indole ring with an isoprene unit.

Rubratoxins, produced by *P. purpurogenum*, belong to a small group of fungal metabolites called nonadrides (Fig. 1.3). Nonadrides are postulated to be derived from the cyclization of two molecules of a precursor which itself is probably formed from the condensation of an activated acyl derivative and oxaloacetate in a reaction analogous to the citric acid synthetase of the Krebs' cycle.



Rubratoxin B

Fig. 1.3 The nonadrides

1.3.4 Mycotoxicoses

Mycotoxins are characterised by their diversity of biological activity which includes antibiotic activity, phytotoxicity, animal toxicity and an immense array of physiological and pharmacological activities in the mammalian system.

Mycotoxicoses is the diseased state resulting in man or animals from the intake of mycotoxins, some 20 of which are considered to pose a risk to human health as food contaminants (Bullerman, 1986). Mycotoxicoses have distinctive characteristics. The diseases are not transmissable, drugs or antibiotics have little effect on the symptoms, and field outbreaks often occur seasonally or linked to certain climatic conditions. Any outbreak can usually be associated with a specific food or feedstuffs and examination of this, normally reveals signs of fungal activity.

Primary mycotoxicoses are diseases caused by ingestion of foods and feeds which have been contaminated by mycotoxins. It is also possible that mycotoxins may pass through the food chain into animal products such as milk or meat which have not themselves been contaminated by fungal growth. Diseases arising from such sources are referred to as secondary mycotoxicoses (Fig. 1.4).

Since fungal toxins are almost all low molecular weight chemical compounds, hence are unsuspected and insidious in their action. Mycotoxins can be acutely or chronically toxic, or both, depending on the dose and the kind of toxin. In animals, symptoms of acute toxicity include liver and kidney damage, attack on the central nervous system, skin disorders and hormone-like effects. Nerve toxins may induce trembling or death without apparent cause. Skin disorders may be manifest as open lesions or as photosensitivity, while the

manifestation of hormonal effects include abortion in cattle, vulvoginitis in pigs, and a variety of ill-defined disorder such as vomit ing, feed refusal and ill thrift. Toxins producing liver and kidney damage are even more insidious: levels much lower than those producing acute effects are often carcinogenic. Ingested in small quantities in the diet, they can cause cancer in experimental animals long after the time of ingestion. It is probable that man can be affected in the same way.

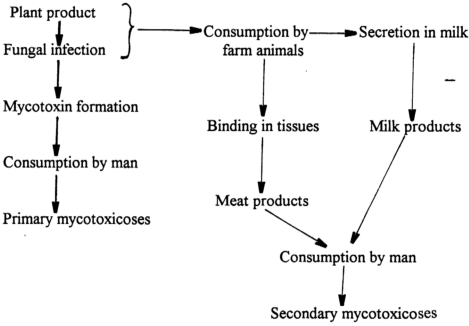


Fig 1.4 Primary and secondary mycotoxicoses

(adapted from Smith and Moss, 1985)

The real crux of human mycotoxicoses comes in the amount of a mycotoxin ingested and the length of time it is present in the food. Acute mycotoxicoses from eating heavily-contaminated food is exceedingly rare in man. However, there is the possibility that low levels can be taken in over long periods,

perhaps intermittently according to the season, with the added hazard that the toxins may well be cumulative.

Austwick (1984) has distinguished human mycotoxicoses into five categories:

i. Historical - disease with good clinical observations, reasonable environmental and epidemiological accounts and fairly convincing evidence.

ii. Actual - disease occurring at the present time with reliable clinical observations e.g. ergot.

iii. Indicative - disease with clinical and mycotoxicological evidence of association with mycotoxins e.g. aflatoxins in Reye's disease.

iv. Speculative - chronic disease of unknown origin which follows the recognized criteria for a mycotoxicoses. Certain types of cancer (e.g. of the breast) come increasingly into this category.

Mycotoxins can enter the system of an animal by ingestion, inhalation or direct skin contact and minute quantities can cause significant health changes when exposed (Smith, 1984; Smith *et al.*, 1984).

1.3.5 Toxicological impact of important mycotoxins

Mouldiness in food products is toxicologically significant, since the fungal species thriving on such products frequently produce toxic secondary metabolites. The consumption of such mouldy products has resulted in mycotoxicoses. It is now well established that mycotoxicoses have been responsible for several major epidemics in man. Human diseases which are now known to be, or are suspected of being caused by mycotoxins are briefly reviewed.

Ergotism occurred throughout the last millenium in Central Europe, and has certainly killed hundreds of thousands of people. By 1750, it was known that ergots results from the growth of the fungus *Clavicep purpurea* (Fr.)Tul. in the ovaries of grains, especially rye. During milling, ergots are not readily separated from sound grain and as a result become fragmented and dispersed throughout the flour. The first symptom of ergotism is a feeling of coldness in the hands and feet, followed by a sensation of intense burning. In extreme cases, gangrene, necrosis and death may follow. The toxic principles of ergots are a range of alkaloids, all derivatives of lysergic acid, which have a wide spectrum of biological activities. The last known outbreak of ergotism occurred in the French village of Pont St. Espritin in 1954. More than 200 people became ill and four died from cardiovascular collapse as a result of muscular spasms. This well-documented mycotoxicosis was due to gross negligence by a miller (Fuller, 1968). Ergotism can now be regarded as a disease of the past since stringent controls on levels of ergot in grain have been established throughout the world.

Another human mycotoxicosis of significance is acute cardiac beriberi. It was a common disease in Japan, especially in the second half of the 19th century. The first symptoms of acute cardiac beriberi are heart distress and palpitation, with rapid breathing. After a few hours, breathing becomes laboured, nausea and vomiting are experienced, and within two to three days, anguish, pain, restlessness and sometimes maniacal behaviour occur. In extreme cases, progressive paralysis leading to respiratory failure may cause death. Beriberi is the general name given to a vitamin B_1 deficiency resulting from the consumption of polished rice.

Uraguchi (1971) established that acute cardiac beriberi probably was not an avitaminosis but a mycotoxicoses.

A third disease caused by a mycotoxin is known as alimentary toxic aleukia (ATA). From 1942 to 1948 this disease caused the deaths of many people in Russia. The symptoms of ATA include fever; haemorrhagic rash; bleeding from the nose; throat and gums; necrotic angina; extreme leucopenia; agranulocytosis; sepsis; and exhaustion of the bone marrow. Outbreaks of ATA were always associated with bread and other cereal products made from grain that was allowed to remain unharvested over the winter months. Russian studies in the 1950s suggested that fungi may have been involved in ATA and that alternate freezing and thawing of the grain was essential for toxin production. During the 1970s it became clear that ATA was a mycotoxicosis caused by the trichothecene toxin known as T-2. Apart from ATA, trichothecenes are involved in a variety of diseases of man and domestic animals. Most of these have occurred in Europe, the USSR, Japan and the United States (Marasas *et al.*, 1979). Toxicity is usually acute, but it has now been suggested that trichothecenes may be implicated in the high incidence of oesophageal cancer that occurs in South Africa (Marasas *et al.*, 1979).

Pellagra is a skin disorder, accompanied by severe mental disturbance and is almost entirely confined to people who subsist on maize of poor quality. Concon (1988) presents evidence that it is due to the growth of *Fusarium* species on moist corn with the consequent formation of T-2 and other trichothecene toxins. During July-Sept 1987, an outbreak of gastrointestinal disease which affected 50,000 people in and around the city of Srinagar, Jammu

and Kashmir, India was attributed to the consumption of bread made from mould-damaged wheat. 39 out of 150 families suffered from the disease with symptoms of abdominal pain (100%), throat irritation (63%), diarrhoea (39%) and vomitting (7%) with onset from 15 mins to 1hr after consuming wheat products. Evidence of mould damage included the presence of *Fusarium* sp. and varying quantities of deoxynivalenol (vomitoxin), nivalenol and T-2 toxin in the wheat samples tested (Bhat *et al.*, 1989).

In Taiwan, 26 persons were poisoned following consumption of mouldy rice for up to 3 weeks. Two samples of mouldy rice contained approximately 200 ug/kg aflatoxins. Three poisoned children died. The victims suffered oedēma of the legs, abdominal pain, vomitting and palpable liver, but no fever (Ling, 1967). In Uganda, a 15-year old boy died of symptoms resembling the cases in Taiwan following consumption of diet which included mouldy cassava. The cassava sample contained 1.7 mg aflatoxin/kg. The autopsy findings revealed liver necrosis and mild fatty liver in addition to other pathological changes (Serck-Hanssen, 1970).

A case of Reye's syndrome reported from Thailand may have involved aflatoxin poisoning (Bourgeois *et al.*, 1971). This case involved a 3-year old who died of Reye's syndrome following consumption of mouldy rice containing as much as 10 mg total aflatoxins/kg. Reye's syndrome is characterized by vomitting, hypoglycaemia, convulsions, hyperammonemia, coma and other acute symptoms. Autopsy revealed cerebral edema and fatty accumulation in the liver cells, tubular epithelium and the myocardial fibers. Two additional cases of Reye's syndrome in

which aflatoxin was found in the tissues were reported from New- Zealand (Becroft and Webster, 1972).

A case can be made regarding the association between liver cancer in man and the consumption of aflatoxin-containing foodstuffs, based on surveys from many parts of the world. In India, aflatoxin consumption may be a factor in the etiology of prevailing childhood cirrhosis, characterized by fatty infiltration of liver cells, leading to cellular degeneration, fibrosis and hepatomegaly (Robinson, 1967). Kwashiorkor patients in India who were unintentionally fed peanut protein supplements contaminated with up to 300 μg aflatoxin/kg developed symptoms similar to those in childhood cirrhosis (Amla, 1971).

In Swaziland, Keen and Martin (1971) found an association between the crude liver cancer incidence and the frequency of contamination of peanut samples. Thus, in the high, middle, and low veld regions where the rates of detectable aflatoxin contamination of peanut samples were 20, 57, and 60% respectively, the corresponding crude liver cancer incidences were 2.2, 4.0 and 9.7 cases/100,000/year. In Uganda, the highest incidence of liver cancer (15 cases/100,000/year) was found to be in the tribal regions where the frequency of aflatoxin contamination of staple foodstuffs was also the highest (44% of the samples collected) (Alpert *et al.*, 1971). About 3.7% of 480 samples contained as much as 1 μgml^{-1} aflatoxin. Studies of aflatoxin consumption and liver cancer incidence in Kenya also have shown a positive correlation (Peers and Linsell, 1972). In three regions of Kenya, the calculated daily intake of aflatoxin, based on estimated consumption of contaminated food and beverages was 4.9, 7.8, and 14.8 $ngkg^{-1}$ body weight. The corresponding liver cancer incidences in these

regions were 3.1, 10.8, and 12.1 cases/100,000/year. In northern Thailand there appears to be a correlation between the incidence of liver cancer and Reye's syndrome (Shank *et al.*, 1972). Many food products which appeared wholesome and fit for human consumption contained relatively high levels of aflatoxin. For example, the aflatoxin content of dried fish, dried chillie peppers, corn and peanuts were shown to be 772, 996, 2700 and over 12,000 p.p.b. respectively. The liver cancer incidences in these regions range from 2 - 6 cases/100,000/year.

Although the actual intake of aflatoxin appears to be quite small, one should remember the extreme carcinogenic potency of aflatoxin B_1. A dose of 1 p.p.b. of aflatoxin is carcinogenic to the Fischer rat (Wogan., 1974).

While the above studies do not warrant a definite conclusion regarding the carcinogenicity of aflatoxin in humans, the consistency of the results in diverse locations and different populations suggest a causal relationship. Because of the extreme carcinogenic potency of aflatoxin in animal species, the widespread distribution of aflatoxin-producing fungi and their ability to grow in different foods grown or stored under various condition, it is highly probable that this mycotoxin poses a serious carcinogenic hazard to humans (Stoloff, 1989).

1.4 MYCOTOXINS PRODUCED BY SPECIES OF *PENICILLIUM* AND *ASPERGILLUS* OCCURRING ON CEREALS

Being the most important filamentous fungal genera invading cereals in storage, a large number of *Penicillium* and *Aspergillus* species have been reported from such commodities (Tuite and Christensen, 1957; Flannigan, 1969, 1970, 1974, 1978; Christensen and Kaufman, 1974; Wallace and Sinha, 1975 and Mills,

1990). However, recent reports show that at normal storage conditions relatively few species of *Penicillium* and *Aspergillus* are important (associated mycoflora) in each food or feed commodity (Frisvad and Filtenborg, 1988).

Pitt (1975) reported that all of the xerophilic species so far reported to be mycotoxic belongs to *Aspergillus* (including *Eurotium* and *Emericella*) and *Penicillium*. Table 1.3 shows the important cereal-borne Aspergilli and their production of known mycotoxins in pure culture. The relative importance of these different species depends on the type of cereal, the occurrence of filamentous fungi in the particular geographic region (which again depend primarily on the climate, i.e. temperature and water activity), storage conditions and possible processing such as acid treatment or controlled atmosphere.

Aspergillus are more commonly associated with the spoilage of foods and other materials in the tropics and warmer climates. This genus is of particular importance because it contains many species capable of growth and metabolism at low A_w and are thus associated with the spoilage of food materials which are too dry to be attacked by other microorganisms.

Penicillium species are common spoiling organisms of cereals, especially in the temperate regions of the world. Unfortunately, *Penicillium* species have seldomly been identified to species level in reports on the mycoflora of cereals. A large number of *Penicillium* species have been reported in the few detailed mycological studies of the *Penicillium* mycoflora of cereals (i.e. Mislivec and Tuite, 1970 and Hill and Lacey, 1984). Species commonly reported from cereals and their mycotoxins are listed in Table 1.4. It is not yet known if all these

mycotoxins are produced under natural conditions, but some of them have been found occurring on cereals.

Table 1.3 Potential mycotoxin production by important species of *Aspergillus* and teleomorphs occurring on cereals

(Adapted from Frisvad and Samson, 1991)

Aspergillus species	Potential mycotoxins
A. candidus	terphenylin, xanthosin
A. clavatus	cytochalasin E, patulin and tryptoquivalins
A. flavus	aflatoxin B_1 (and B_2), aflatrem, cyclopiazonic acid, kojic acid maltoryzin, and 3-nitropropionic acid
A. fumigatus	fumigaclavines, fumigatin, fumitoxins, fumitremorgins and verrucologen, gliotoxin and tryptoquivalins
A. niger	malformins and naphthopyrones
A. nomius	aflatoxin B_1, B_2, G_1, G_2 and kojic acid
A. ochraceus	ochratoxin, penicillic acid, secalonic acid A and xanthomegnin and viomellein
A. parasiticus	aflatoxin B_1, B_2, G_1, G_2, kojic acid and 3-nitropropionic acid
A. restrictus	mitogillin
A. tamarii	cyclopiazonic acid, fumigaclavine A and kojic acid
A. terreus	citreoviridin, citrinin, patulin and territrems
A. versicolor	nidulotoxin and sterigmatocystin
Emericella nidulans	nidulotoxin and sterigmatocystin
Eurotium amstelodami	physicons
Eurotium chevalieri	physicons
Eurotium repens	physicons
Eurotium rubrum	physicons

Table 1.4 Potential mycotoxin production by important species of *Penicillium* and teleomorphs occurring on cereals

(Adapted from Frisvad and Samson, 1991)

Penicillium species	Potential mycotoxin
P. aethiopicum	viridicatumtoxin
P. aurantiogriseum var. aurantiogriseum	nephrotoxic glycopeptides penicillic acid, verrucosidin viomellein and xanthomegnin
P. aurantiogriseum var. melanoconidium	penicillic acid, penitrem A verrucosidin, viomellein and xanthomegnin
P. aurantiogriseum var. viridicatum	penicillic acid, viomellein viridic acid and xanthomegnin
P. brevicompactum	botryodiplodin
P. chermesinum	costaclavin, patulin – phoenicin, plastatin and luteosporin
P. chrysogenum	roquefortine C and xanthocillin X
P. citreonigrum	citreoviridin
P. citrinum	citrinin
P. crateriforme	rubratoxin, rugulovasines and spiculisporic acid
P. expansum	chaetoglobosins, citrinin, patulin and roquefortine C
P. griseofulvum	cyclopiazonic acid, patulin and roquefortine C
P. hordei	roquefortine C, terrestric acid
P. islandicum	cychlochlorotine, emodin, erythroskyrine, luteoskyrine and rugulosine
P.oxalicum	roquefortine C and secalonic acid D
P. piceum	rugulosine
P. roqueforti var. carneum	botryodiploidin, patulin, penicillic acid and roquefortineC
P. simplicissimum	xanthomegnins
P. thomii	oxathine carboxylic acid
P. variabile	pevalic acid and rugulosin
P. verrucosum	citrinin, verrucolone and ochratoxin A, B, C

1.5 PRESENT STATUS OF FUNGAL CONTAMINATION AND MYCOTOXIN SITUATION IN MALAYSIA

Food fungi and mycotoxin problems have not by-passed tropical Asian countries. Recent survey has shown the occurrence of *Aspergillus flavus* to be widespread throughout the tropics. The frequency of contamination by mycotoxins is unknown but aflatoxin is frequently detected in a variety of feeds and foods in ASEAN countries.

A report by Lim in 1964 on an outbreak of a disease which had probably occurred in 1960 on two pig farms in Malacca was probably the first suggestion on the occurrence of aflatoxin problem in Malaysia. Gross liver damage was said to be associated with the introduction of feedstuffs containing peanut meal and peanut cake imported from Thailand. Later, Lim and Yeap (1966) reported the detection of aflatoxins in various feed ingredients imported into the country, including several types of oil cakes and meals.

The detection of aflatoxin in food and agricultural produce was first carried out by Institute of Medical Research, Malaysia in 1965 on groundnut and groundnut oil for cooking (Mat Isa and Tee, 1984).

Later a survey for the detection of aflatoxin in foods and agricultural produces was also carried out by the Food Technology Division, Malaysian Agricultural Research and Development Institute (MARDI). During the screening studies carried out in 1981 - 1984, 10 out of 17 samples of raw shelled groundnut was aflatoxin positive with a range from 2 - 400 μgkg^{-1} (Mat Isa and Tee, 1984). In another survey of raw shelled groundnut randomly collected from retail outlets

throughout Peninsular Malaysia in 1985, 88.5% out of 96 samples analysed were aflatoxin positive ($\geq$ 5 μgkg^{-1}) with 53.1% having a level of $\geq$ 40 μgkg^{-1} (Anon, 1985). Local peanut butter was found to contain higher level of aflatoxin as compared to the imported version ($\leq$ 20 μgkg^{-1}).

Allegations that Malaysian pepper contained high microbial loads including the aflatoxin producing species has led to a screening of black and white pepper in 1984 and 1985. All 51 samples of black pepper and 16 samples of white pepper having a moisture content of 9 - 11% obtained from the Pepper Marketing Board (PMB), Kuching, Sarawak were contaminated with aflatoxin. 72.5% black pepper and 62.5% white pepper had a level of $\geq$ 40 μgkg^{-1}. The remaining samples had levels between 5 - 20 μgkg^{-1}. Actions have been taken in the form of reprocessing including recleaning of all pepper intended for export in order to reduce the extent of contamination (Anon,1985; Mat Isa and Nazarifah, 1986).

In 1986, 36 representative samples of dried cocoa beans, having a moisture content between 5.3% - 13.8% were analysed for aflatoxin. 31% of the samples had levels of $\geq$ 35 μgkg^{-1} and the rest $\leq$ 25 μgkg^{-1} (Anon, 1986).

Out of 36 copra samples analysed in 1981 and 1984, 69.4% were found to contain aflatoxin at a level of 4 - 400 μgkg^{-1} and the rest $\geq$ 40 μgkg^{-1} (Anon, 1981; 1984).

One hundred and fifty-five samples of dry and wet spices (e.g. curry powder, 'kurma' powder, dried chillies and 'chillie boh') from 19 different types of commonly used home spices were analysed for aflatoxin. 90.3% was found to have a level of $\geq$ 35 μgkg^{-1} and the remaining had levels of $\leq$ 25 μgkg^{-1}. Contamination was due to their method of storage at retail outlets where most of

them are kept for a long period in open containers and subjected to environmental changes (Anon, 1987).

In the screening of paddy and rice, in 1981 and 1982, six out of 77 samples of paddy were aflatoxin positive with a level of 2 - 8 μgkg^{-1}. All 22 samples of stored rice however, were free of aflatoxin (Anon, 1982).

In a report by Saito and Singh (1976), a study of mycotoxins in foods in relation to liver diseases in Malaysia showed that the incidence of liver cancer is higher in Malaysia than in Japan. Among the three races (Malays, Chinese and Indians), the incidence of liver cancer is highest in the Chinese male population. They indicated that this may be due to the type of diet of each race. Included in their report was a mycological survey of Malaysian foods and *Aspergillus* spp. were the major fungi found. Of 30 strains of *A. flavus* isolated, 8 produced aflatoxin and 3 of 4 strains of *A. versicolor* (Vuill.)Tiraboschi produced sterigmatocystin. All 91 strains of *A. candidus* Link examined produced terphenyllin but not xanthoascin.

An outbreak of acute hepatic encephalopathy involving mainly children occurred in the state of Perak in 1988. This was the first known outbreak ever to occur in Malaysia. During this mishap, 13 Chinese children and an elderly woman were admitted after consumption of contaminated Chinese noodle, loh shee fun. Loh shee fun is processed from several types of raw materials consisting of rice, cornflour, tapioca flour and wheat starch. All children died and symptoms suffered by victims are stomach ache, vomitting and diarrhoea. Postmortem samples from patients showed a very high concentration of aflatoxin B_1, aflatoxin B_2 and aflatoxin G_1 in the liver, kidney, lung and brain samples (Lye *et al.* 1995).

Confirmation of the presence of mycotoxins other than aflatoxin in the raw materials from the factory suspected was not done. (See Appendix C for reports of the incidence).

In the aetiology of food poisoning outbreaks reported in Malaysia in 1988, the highest percentage of deaths was due to fungal contamination namely, aflatoxin i.e. 14 out of 17 cases (Toxins in Food Seminar, 1989).

Since the problem of aflatoxin was only realised in 1960's, therefore no mention was made in the Sale of Food and Drug Ordinance, 1952 which governed the food regulations in this country prior to 1985. However, the regulations on aflatoxin in foods from other countries such as the U.K. and U.S.A. was used as a reference. With the introduction and implementation of the Food Regulations, 1985 through the Food Act 1983, the permissable level of aflatoxins in foods is specially mentioned. Table II of the fifteenth schedule (Regulation 39) mentioned that the maximum permitted proportions of mycological contaminant in the proportions of mycological contaminant (aflatoxin or any other mycotoxin) is 35 μgkg^{-1}. This regulation stipulated that there shall be no importation, preparation or advertisement for sale or sell any food which contains the mycological contaminant in the proportion greater than the value specified in the Table. The monitoring of mycotoxins is included under 'Food Quality Monitoring Programme" conducted by the Ministry of Health.

1.6 SCOPE AND OBJECTIVES OF STUDY

From the published reports discussed above, it is evident that food spoilage and mycotoxin contamination poses a serious problem to man and animals. Humans are exposed to mycotoxins by consuming foods contaminated with products of fungal growth. Such exposure is difficult to avoid because fungal growth in foods is not easy to prevent.

Malaysia is situated between latitudes 1° N to 7° N and longitudes 100° E to 119° E. It has an equatorial climate with high uniform temperature, high humidity and copious rainfall. Malaysia has a mean temperature of 26.2 °C with mean max. of 31.8 °C and mean min. of 22.8 °C; mean relative humidity of 85.6% with mean max. of 98.9% and mean min. of 60.4%. Under conditions of high ambient humidities, control of stored commodity moisture is difficult and become very susceptible to fungal contamination and spoilage. Together with improper storage methods and marketing conditions, problems in maintaining an adequately low A_w occur and there is a risk of mycotoxin contamination in foods. In Malaysia, work on mycotoxin contamination has been concentrated on aflatoxins (Hew Voon Fong and Hutagalung, 1977 and Mat Isa and Tee, 1984). No study was carried out on the effect of storage conditions on fungal growth and production of other mycotoxins.

Food is a rich habitat for the growth of any spoilage microorganism, containing an abundance of nutrients such as carbohydrate, proteins, lipids and growth factors like vitamins and minerals. Generally, it appears that fungal metabolism is best suited to substrates high in carbohydrates, while bacteria with

Lactobaccilli as an exception, are more likely to spoil proteinaceous foods (Pitt and Hocking, 1985).

Hence, considering the reasons mentioned above, the present study was carried out to determine the status of fungal contamination and to shed some light into the effects of A_w on fungal growth and hence, mycotoxin production in starch-based food namely, rice, glutinous rice, riceflour, glutinous riceflour, wheatflour and cornflour. Rice is an important staple food in the Malaysian diet and the other cereal products are frequently used as raw materials for the preparation of processed foods.

Hence, the present study was undertaken with the following aims:

i. To carry out a survey on the fungal count and the incidence of aflatoxigenic species on starch-based food in Malaysia and to conduct a survey on the presence of aflatoxins in rice and wheatflour sampled at the consumer level in Malaysia.

ii. To determine the water adsorption isotherms of starch-based food and its relationship to fungal development.

iii. To investigate the incidence of fungal species in starch-based food during storage at different levels of water activity and their participatory roles in the biodeterioration of starch-based food. A key for identification and taxonomic studies of the fungi isolated on starch-based food were also included in this study.

iv. To evaluate an extraction and detection method of mycotoxins in starch-based food using the reversed-phase high-performance liquid chromatography (HPLC). The quantitation of mycotoxins present in

starch-based food when stored at different levels of water activity were also carried out. The toxicity of starch-based food extracts were also tested on the brine shrimp, *Artemia salina L.*