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REVIEW ARTICLE

Fungi, quality and safety issues in fresh fruits and vegetables

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Abstract

The role of moulds in the spoilage of fresh fruits and vegetables is discussed. Although the major problems are economic with a significant loss of useful food materials, there are a few examples implicating a role for mycotoxins in the safety of fresh fruits. The significance of the mycotoxins patulin, ochratoxin and tenuazonic acid will be reviewed.

Introduction

Despite the high water activity of most fruits and vegetables, the low pH, especially of fruits, gives fungi a competitive advantage over the majority of bacteria and mould spoilage is not uncommon. However, the plant and fungal kingdoms have evolved together and many plants have endophytic and mycorrhizal fungi associated with them. In a healthy plant these relationships may be beneficial to both partners and, in addition, plants have evolved many mechanisms to prevent overt fungal attack of the living tissue. These may involve physical barriers, chemical barriers or, at a more sophisticated level, the production of antifungal metabolites, known as phytoalexins, in response to fungal attack.

An interesting example of phytoalexin production which may have health implications is the production of metabolites such as ipomeamarone and 4-hydroxymyoporone by the sweet potato (*Ipomoea batata*). These are hepatotoxins to mammals as well as having antifungal activity. Furthermore they can be broken down by a few moulds, such as *Fusarium solani*, to compounds such as 4-ipomenol which may cause oedema of the lung (Fig. 1). A number of species of *Penicillium* and *Aspergillus* produce mycotoxins such as patulin and ochratoxin A for which many countries have set limits in foods for human consumption. Patulin is especially important in apple juice and apple products and, although ochratoxin A is predominantly a problem in commodities such as cereals, cocoa and coffee, it is also found in grapes and grape products.

Fungal spoilage of fruits and vegetables

In dealing with fresh fruits and vegetables as food materials it is important to recognize that they are living tissues and fungal invasion of these tissues requires that the fungi overcome the many barriers that plants have evolved to keep them at bay. This often means that there is a degree of specificity between a particular commodity and the mould species which have overcome these barriers to produce overt spoilage. The low pH of most fruits, as low as 2·2 in the case of lemons, leads to the spoilage of this group of plant products being predominantly by fungi. Vegetables, in contrast, have pH values closer to neutrality (4·8–6·5) and, as well as fungi, bacteria play a significant role in their spoilage. Those genera and species of fungi associated with the spoilage of fruits and vegetables are extensively described by Pitt and Hocking (1997) and Snowden (1990, 1991).

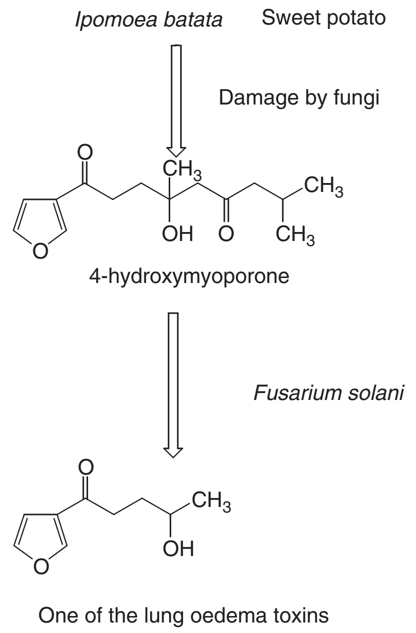

Figure 1 Toxic metabolites from *Ipomoea*.

In general, mould spoilage of fruits and vegetables does not lead to a health hazard because the commodities are usually rejected but the economic losses may be considerable. Before dealing with the few examples where health issues may be a concern, I would like to highlight a small number of mould species which can have a major role in the spoilage of fresh plant commodities.

Botrytis

It is difficult to believe that anyone who enjoys fresh fruits, especially strawberries, has not met grey mould (*Botrytis cinerea*). The hyaline tip of the dark, almost metallic, conidiophores branches repeatedly and eventually each branch tip swells and they all synchronously produce a mass of colourless blastospores which are readily dispersed by wind and insects. This species of *Botrytis* can also produce sclerotia and, when two compatible mating types come together, a stalked apothecium of the ascomycete sexual stage *Sclerotinia* (*Botryotinia*) *fuckeliana*. This life cycle and the biology of this mould are clearly described by Webster and Weber (2007) and Weber and Webster (2003). *Botrytis cinerea* has a wide host range and may cause spoilage of fruits (e.g. raspberries, strawberries, grapes, kiwi fruit, pears, peaches, plums and cherries) as well as vegetables (e.g. carrots, lettuce, peas and beans), indeed it is reported to be pathogenic to more than 200 species of plants.

Although generally a spoilage organism of grapes there is one situation in which *B. cinerea* enhances the value of this commodity giving rise to what is known as the 'noble rot' when the infection is milder and the grapes dry out slowly becoming richer in fermentable sugars and flavours. These grapes are then used in the production of high-quality dessert wines.

In contrast to *B. cinerea*, *Botrytis allii* is a specific pathogen which causes spoilage of onions and related crops such as garlic. A monograph of the genus, its biology, pathology and control has been produced by Elad *et al.* (2004).

Penicillium

The genus *Penicillium* is an important and extensively described genus (see Pitt 1979) with both beneficial and spoilage species. Among the most commonly encountered species causing spoilage of fruit are *Penicillium italicum*, associated with the blue rot of citrus, *Penicillium digitatum* causing a devastating and rapid green rot of citrus, and *Penicillium expansum* the blue rot of apples and pears. Apart from the possibility of allergic response to the enormous numbers of dry air-borne spores produced by *P. italicum* and *P. digitatum*, especially the latter, there are no special health risks associated with these species as the rotten fruit will usually be discarded. This is not always the case with *P. expansum* which produces the mycotoxin patulin.

Unlike *B. cinerea*, penicillia do not usually attack grapes before harvest but may do so during storage. The most commonly encountered species is *P. expansum* but other species such as *Penicillium aurantiogriseum* and *Penicillium chrysogenum* may also be encountered. Species of *Penicillium* are not often associated with fresh vegetables but some, such as *Penicillium glabrum* and *Penicillium funiculosum*, may cause spoilage and disease of onions.

Rhizopus and Mucor

Some species of these well-known members of the Zygomycota are frequently associated with the spoilage of fresh fruits and vegetables. *Rhizopus stolonifer* and *Mucor piriformis* are responsible for the rapid decay of soft fruits such as raspberries and loganberries and these moulds can spread rapidly especially on commodities stored at temperatures above 20°C. *M. piriformis* has been described as a destructive pathogen of strawberries (Snowden 1990, 1991) and a number of species of *Rhizopus*, especially *Rhizopus sexualis*, are pathogenic to strawberries causing a soft rot (Harris and Dennis 1980). The author has personal experience of a soft rot of apples in store caused by a species of *Mucor* and *M. piriformis* has been reported as a problem in cold-stored apples and pears (Caccioni and Guizzardi 1992).

Both *Rhizopus* and *Mucor* can cause serious losses of tomatoes. Initially attack may be through a damaged part of a single fruit involving a small inoculum but, once a single fruit has become extensively infected, the active mycelium and large numbers of spores formed are able to invade healthy intact fruit and the moulds can spread very rapidly through a consignment. This phenomenon has been referred to as 'the inoculum effect'.

Mycotoxin problems in fruits and vegetables

The major crops associated with the most significant mycotoxins, such as aflatoxins, trichothecenes and fumonisins, are cereals, groundnuts and treenuts and they are not normally a problem in fresh fruits and vegetables. There are, however, a few exceptions and undoubtedly the most important is patulin. Perhaps more important toxicologically, but far less widespread in fresh fruits and vegetables is ochratoxin and perhaps a case can be made for including tenuazonic acid.

Patulin

Patulin (Fig. 2) is one of the smallest of the group of toxic metabolites known as polyketides. It may be produced by a number of species of *Aspergillus*, *Penicillium* and *Paecilomyces* but by far the most important species in the context of fruits and fruit products for human consumption is *P. expansum*. The biosynthesis of patulin occurs over a much narrower range of a_w than growth of the mould and it is most stable at low pH so the environment of fresh fruit is ideal for its production. *Penicillium expansum* is especially associated with a rapid soft rot of apples and will eventually produce characteristic rings of blue pustules of spores giving this disease its common name of 'blue rot'.

In 1993 a newspaper headline announced an 'Apple Juice in Cancer Scare' based on the discovery that 5/42 samples of apple juice taken from shops contained more than 50 μg kg^{-1} of patulin which, at that time, was the advisory limit set in the United Kingdom (MAFF 1993). The offending samples were reported to contain 59, 82, 118, 153 and 434 μg kg^{-1} and a review by Pittet (1998) showed that patulin was indeed quite widespread in several countries throughout the world with incidence ranging from 21% to 100% of samples analysed and concentrations ranging from 5 to 1130 μg kg^{-1}.

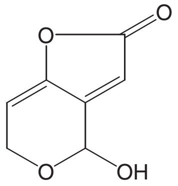

Figure 2 Patulin.

How toxic is patulin? The oral LD$_{50}$ in the rat is reported to be 32·5 mg kg^{-1} body weight and a 2-year study on Wistar rats gave a no observed effect level (NOEL) of 43 μg kg^{-1} body weight. It is very rapidly excreted and 87% of a single dose is excreted in the faeces (49%), in urine (36%) and respired as carbon dioxide (1–2%). Patulin was first discovered as an antibiotic with a wide spectrum of activity but was soon proved to be too toxic to be used clinically. It has also been shown to have some immunosuppressive activity (Sharma 1993) and this, combined with its antibiotic activity, could be of concern in some clinical situations. There is no evidence of carcinogenic activity when consumed orally and the main source of patulin in the human diet is apple juice made using apples which include some moulded by *P. expansum*. The fruit itself presents little risk as the mouldy portion will normally be discarded. Indeed, if overtly mouldy apples can be rejected from a batch used for preparing juice, levels of patulin can be kept very low. Unfortunately it is not always possible to detect the presence of *P. expansum* from the external appearance of the fruit because, in some varieties with an open core structure, infection can be initiated inside the apple. Although Jackson *et al.* (2003) reported patulin accumulating in apples during cold storage in their studies of the influence of apple quality, storage and washing treatments on patulin production, Morales *et al.* (2007) demonstrated that cold storage at 1°C for 6 weeks did not lead to detectable levels of patulin. They used apples inoculated with *P. expansum* in these experiments and found that, on subsequent storage for a further 3 days after 6 weeks in cold storage, patulin could be detected.

Patulin is relatively stable at pasteurization temperatures (e.g. 10 s at 90°C) so this process will not reduce levels of patulin sufficiently to make them acceptable (Harrison 1989) if the juice is badly contaminated before pasteurization. During the fermentation of apple juice to cider using the yeast *Saccharomyces cerevisiae* patulin is degraded to ascladiol (Moss and Long 2002), the presence of which in cider would provide an indicator of the use of poor quality fruit in its production. As apple juice is so widely consumed it seemed sensible to set limits to the levels of patulin in this commodity and in March 2004 the European Community (EC) set a limit of 50 μg kg^{-1} for all fruit juices, 25 μg kg^{-1} for solid apple produce used for direct consumption and 10 μg kg^{-1} in apple juice and apple products for babies and young children (EC no. 455/2004). Legislation requires that there be reproducible analytical procedures of appropriate sensitivity and these are reviewed by Roach *et al.* (2002).

Ochratoxin A

The major source of ochratoxin A (Fig. 3) in the diet is from cereals which would be contaminated by *Penicillium verrrucosum* in temperate climates or *Aspergillus ochraceus* and related species in warmer parts of the world. Species of *Aspergillus* within the section *Circumdati* have been studied intensively using morphological, molecular and biochemical characters leading to the description of seven new species (Frisvad *et al.* 2004). The isolate, originally described as *A. ochraceus*, from which ochratoxin was first discovered in South Africa, is now the type strain of *Aspergillus westerdijkiae* (Frisvad *et al.* 2004) and this species is now recognized as a major producer of ochratoxin A. Some strains of *A. ochraceus* do produce ochratoxin A but it is a variable character in this species. Those species of *Aspergillus* with yellow- to ochre-coloured spores (section *Circumdati*) consistently producing ochratoxin A include *Aspergillus cretensis, Aspergillus flocculosus, Aspergillus pseudoelegans, Aspergillus roseoglobulosus, Aspergillus sulphurous* and *Neopetromyces muricatus*, as well as *A. westerdijkiae*.

During the workshop of the International Commission on Food Mycology held in June 2007, *A. westerdijkiae* was frequently discussed as a dominant producer of ochratoxin A, especially associated with coffee beans and cocoa products (http://www.foodmycology.org). However, ochratoxins can also be produced by some members of the black-spored *Aspergillus niger* group particularly *Aspergillus carbonarius*. These species cause bunch rots of grapes and ochratoxin has been detected in both the fresh fruits and raisins as well as in wine and grape juice. Bellí *et al.* (2004) demonstrated the production of ochratoxin A by a number of strains of *A. carbonarius* and *A. niger* isolated from grapes and grown in a medium similar in composition to grape juice. They demonstrated that both a_w and time of incubation influenced the concentration of ochratoxin A produced. Although one strain (W120) of *A. carbonarius* could produce ochratoxin A at a_w as low as 0·90, the optimum a_w was 0·98 with maximum production at 5 days. Longer incubation led to a reduction in ochratoxin A and it is suggested that this could be because of the ability of the mould to break down the mycotoxin to compounds such as ochratoxin α with highly reduced toxicity (Varga *et al.* 2002).

Ochratoxin A is efficiently absorbed by the body, has nephrotoxic properties and, unlike patulin, it has a long residence time in body tissues, thus it is detectable in the blood of mice 18 days after receiving a single dose. Being a derivative of L-phenylalanine, it is a potent inhibitor of the incorporation of this amino acid into proteins by inhibiting phenylalanine t-RNA synthetase. It also inhibits the biosynthesis of tyrosine by inhibiting phenylalanine hydroxylase.

Ochratoxin A has immunosuppressive activity but, most importantly, it is carcinogenic in some animal species and it is classified as a possible human carcinogen by the International Agency for Research on Cancer (IARC). In March 2002 the EC set the following limits for ochratoxin A; in raw cereals, 5 $\mu g\ kg^{-1}$; cereal products, 3 $\mu g\ kg^{-1}$; and dried vine fruit, 10 $\mu g\ kg^{-1}$ (EC no. 472/2002). By September 2004, this list of commodities was extended to include roasted coffee (5 $\mu g\ kg^{-1}$) and soluble coffee (10 $\mu g\ kg^{-1}$) and limits of 2·0 $\mu g\ kg^{-1}$ for wine and grape juice were set in 2005. Clearly such stringent limits require sensitive, specific and reproducible analytical methods and clear sampling plans for a wide range of food commodities. Such methods for sampling and analysis of ochratoxins have been reviewed by Scott (2002).

The importance of ochratoxin A in food is reflected in the Proceedings of a Workshop held in 2005, organized by ILSI Europe, and published in *Food Additives and Contaminants* (Suppl. 1), 2005, pp. 1–107. Specific concern about the occurrence of ochratoxin A in grapes and wine is extensively reviewed in a special supplement of the *International Journal of Food Microbiology* (Battilani *et al.* 2006).

Tenuazonic acid

The genus *Alternaria* has numerous species many of which are described in detail by Ellis (1971, 1976). They

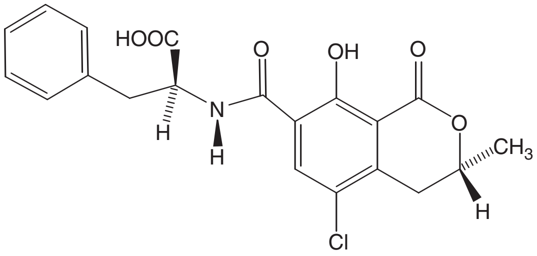

Figure 3 Ochratoxin A.

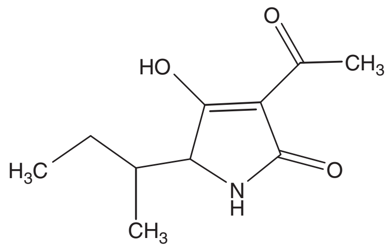

Figure 4 Tenuazonic acid.

are generally associated with plants and plant products both as pathogens and saprophytes and many of them are very host-specific. *Alternaria alternata* (= *A. tenuis*), however, has a wide host range as a pathogen and can occur as a saprophyte on a range of substrates such as foodstuffs and textiles. The genus is associated with the biosynthesis of a diverse range of secondary metabolites, many of which have biological activity. Many aspects of this activity are reviewed in detail by Bottalico and Logrieco (1998) who also provide an extensive bibliography.

In the context of fresh fruits and vegetables for human consumption *Alt. alternata* is especially important. It is able to grow on a wide range of fruits and vegetables and is a major pathogen of fresh tomatoes in which it can produce tenuazonic acid (Fig. 4). Mislivic *et al.* (1987) reported on the significance of tenuazonic acid in fresh tomatoes used for the production of Catsup (Ketchup or tomato sauce).

Unlike patulin and ochratoxin A there are no regulatory limits set for tenuazonic acid or any other *Alternaria* metabolites, such as the alternariols, reflecting the lack of any evidence implicating them in human illness.

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