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Review

Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods

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ABSTRACT

Food decay by spoilage fungi causes considerable economic losses and constitutes a health risk for consumers due to the potential for fungi to produce mycotoxins. The indiscriminate use of synthetic antifungals has led to the development of resistant strains which has necessitated utilization of higher concentrations, with the consequent increase in toxic residues in food products. Numerous studies have demonstrated that plant extracts contain diverse bioactive components that can control mould growth. The metabolites produced by plants are a promising alternative because plants generate a wide variety of compounds, either as part of their development or in response to stress or pathogen attack. The aim of this article is to summarize the results from the literature on *in vitro* and *in vivo* experiments regarding the effects of plant-derived products for controlling fungal growth. Data from research work on the mode of action of these metabolites inside the fungal cell and the influence of abiotic external factors such as pH and temperature are also covered in the present review. Furthermore, an analysis on how the stress factor derived from the presence of plant extracts and essential oils affects secondary metabolism of the fungus, specifically mycotoxin synthesis, is developed. Finally, the effectiveness of using plant-derived compounds in combination with other natural antimicrobials and its application in food using novel technologies is discussed.

Contents

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1. Introduction

Numerous pests and diseases attack food crops around the world; most of them are related to pathogenic fungal diseases. Worldwide, post harvest losses have been estimated at 50% and much of this is due to fungal and bacterial infections (Magro et al., 2006). Moulds are ubiquitous biological agents that are able to colonize foods because of their potential to synthesize a wide diversity of hydrolytic enzymes. They cause pathologic disorders in plants bringing considerable economic losses for food producers.

Fruits and vegetables are highly susceptible to fungal spoilage, both in the field and during postharvest storage. Significant genera include *Pythium*, *Phytophthora*, *Fusarium*, *Penicillium*, *Alternaria*, *Botrytis*, *Geotrichum*, *Sclerotinia* and *Rhizoctonia* spp. Fungal growth on fresh fruits and vegetables is responsible for food spoilage and numerous plant diseases, which lead to significant economic losses. Mould growth depends on abiotic factors such as pH, water activity (a_w), solute concentration, temperature, atmosphere, time, *etc.* However, conditions of temperature and a_w are the main variables determining the development of fungi. Grain crops are also vulnerable to fungal contamination, with *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* being the most frequent genera. In this matrix, moulds are responsible for off-flavor formation and contribute to heating and loss in dry matter in grains through the utilization of carbohydrates as an energy source, degradation of lipids and proteins, production of volatile metabolites and production of allergenic compounds. This causes a reduction in the quality of animal feed and seed (Magan and Aldred, 2007). These events can take place even before the fungal growth is evident (Lee et al., 2007).

Apart from causing diseases in plants, many species of *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria* can also synthesize mycotoxins. These compounds are hazardous to animal and human health as they can be lethal, carcinogenic, mutagenic, teratogenic, immunosuppressant, or may mimic estrogens. Their activity depends on the type of toxin and their concentration in the food. Concern about these chemical hazards has been increasing due to the wide range of food types that may be affected and the variability in the severity of symptoms caused. The presence of mycotoxins in food is associated with fungal inoculum on predisposed substrates. Mycotoxins can be produced before and after harvest and levels may increase during postharvest handling and storage. Thus prevention of fungal growth is an effect means of preventing mycotoxin accumulation. Mycotoxins may reach consumers either by direct contamination of plant materials or products thereof, or by 'carry over' of mycotoxins and their metabolites into animal tissues, milk and eggs after intake of contaminated feed. Furthermore, this hazard remains in processed food because these metabolites are not removed by normal industrial processing, and the risk could increase if mouldy fruits or plants are used in processed byproducts.

Inhibition of fungal growth in crops, fresh fruits and vegetables is thus necessary to reduce the risk to human and animal health. However, it is important to note that partial inhibition of fungal growth, such as reduction of fungal growth rate, could enhance mycotoxin production as a response of the mould to stress.

2. Drawbacks of synthetic fungicides

The first step in fighting fungal contamination is the application of fungicides in the field. Fungitoxicants can be applied postharvest, provided they do not adversely affect the appearance or quality of the treated commodities (Amiri et al., 2008). Antimicrobial chemicals such as benzimidazoles (*e.g.* thiabendazole), aromatic hydrocarbons (*e.g.* sodium ortho-phenylphenate) and sterol biosynthesis inhibitors (*e.g.* imazalil, a sterol demethylation inhibitor) have been used for decades in control of plant diseases in agriculture. More recently, two other fungicides, each with different mode of action, have become important in the market: pyrimethanil (anilinopyrimidine) and fludioxonil (phenylpyrrole). The indiscriminate and excessive use of fungicides in crops has been a major cause of the development of resistant pathogen populations, resulting in the use of higher concentrations of these antifungals and the consequent increase in toxic residues in food products. For example, acquired resistance by *Penicillium italicum* and *P. digitatum* to many synthetic fungicides currently used on citrus fruit has been demonstrated (Fogliata et al., 2001). Some of these compounds are not biodegradable, so they can accumulate in soil, plants and water, and consequently affect humans through the food chain. Although chemical treatments have been considered to be the cheapest and most effective way to prevent postharvest diseases, the development of resistant microorganisms has reduced their acceptance. The type and concentration of fungicides allowed for postharvest application are restricted due to their long degradation period and potential effects on food and human health (carcinogenicity, teratogenicity, high and acute residual toxicity, hormonal imbalance and spermatotoxicity). Because of these undesirable effects, recent studies resulted in the revocation of registration of some of the more effective fungicides. Furthermore, public concern about food contamination with fungicidal residues has significantly increased. Considering all these factors, the development of new safe and biodegradable alternatives that are both effective and economically feasible is needed.

3. Low impact chemical preservative agents

In recent years, consumers' preferences are moving towards foods that contain lower levels of chemical preservatives and exhibit more fresh-like and natural characteristics. The salts of weak acids, such as sodium benzoate and potassium sorbate, can inhibit growth of several postharvest fungal pathogens. Using these compounds for fungal inhibition presents several benefits, such as their low mammalian toxicity, a wide spectrum of activity and relatively low cost. However, high concentrations of these compounds are needed to act as fungicides, bringing associated potential organoleptic changes. For example, calcium propionate completely inhibited mycelial growth of *Botrytis cinerea* at a level of 5% (w/v) (Droby et al., 2003). Benzoic acid, one of the most broadly used antimicrobials, is permissible at levels up to 0.1% (Jay, 2000). It is commonly applied in the form of benzoates, mainly as sodium benzoate, due to the higher solubility of the salts. In general, optimal inhibitory activity takes place at low pH since acid conditions favor the undissociated form of the molecule that freely crosses the plasma membrane of the target cell. Inside the cell, the molecule will dissociate due to a higher pH; the preservative action is thought to be due to an accumulation of anions and protons inside the cell (Brul and Coote, 1999; Jay, 2000). Cytoplasmatic pH decrease due to the entry of the undissociated state of the compound may cause the rupture of certain metabolic reactions of the microorganism, leading to the permeabilization of the cytoplasmic membrane and cell death. Other compounds frequently used for their fungistatic activity are the antioxidants butylated hydroxyanisole (BHA), propyl paraben (PP) and butylated hydroxytoluene (BHT). Like benzoic acid, they are considered Generally Recognized As Safe (GRAS) by the US Food and Drug Administration (FDA), which also allows their use as antimicrobial agents in food. The Codex Alimentarius (2006) established the maximum usage level for single or multiple antioxidants as 200 μg/g based on the weight of the fat or oil. Furthermore, nisin, monolaurin, and lactoperoxidase are examples of "natural" preservatives, but they have several limitations, which include limited spectrum of activity, high application costs, the potential emergence of resistant strains and their impact on the organoleptic properties of foods.

The application of the hurdle technology concept, the utilization of several preservative factors (hurdles) together at lower levels, could overcome these constraints. The use of naturally occurring antimicrobial compounds in combination with mild food processing treatments and chemical additives have been widely studied recently. The treated